12 • The opium analgesics

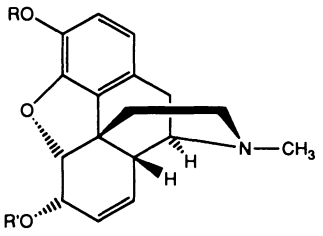
12.1 *Introduction*

We are now going to look in detail at one of the oldest fields in medicinal chemistry, yet one where true success has proved illusive—the search for a safe, orally active, and non-addictive analgesic based on the opiate structure.

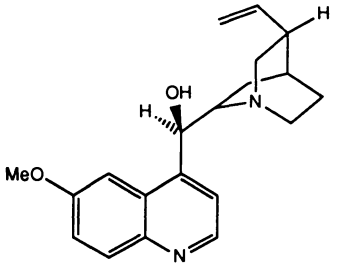
It is important to appreciate that the opiates are not the only compounds which are of use in the relief of pain and that there are several other classes of compounds, including aspirin, which combat pain. These compounds, however, operate by different mechanisms from those employed by the opiates, and therefore relieve a different, 'sharper' kind of pain. The opiates have proved ideal for the treatment of 'deep' chronic pain and work in the central nervous system (CNS).

The term 'opium alkaloids' has been used rather loosely to cover all narcotic analgesics, whether they be synthetic compounds, partially synthetic, or extracted from plant material. To be precise, we should really only use the term for those natural compounds which have been extracted from opium—the sticky exudate obtained from the poppy (*Papaver somniferum*). The term alkaloid refers to a natural product which contains a nitrogen atom and is therefore basic in character. There are, in fact, several thousand alkaloids which have been extracted and identified from various plant sources and examples of some of the better known alkaloids are shown in Fig. 12.1. These compounds provide a vast 'library' of biologically active compounds which can be used as lead compounds into many possible fields of medicinal chemistry. However, we are only interested at present in the alkaloids derived from opium.

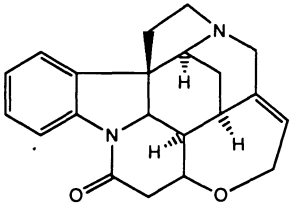
The opiates are perhaps the oldest drugs known to man. The use of opium was recorded in China over two thousand years ago and was known in Mesopotamia before that. Over the centuries the crude extract derived from poppies has been widely used as a sedative. A tincture of opium called laudanum was introduced to England and was considered indispensable to medicine. It is ironic that a compound renowned for its sedative effects should have led to at least one war. In the nineteenth century, the Chinese authorities became so alarmed about the addictive properties of opium that they tried to ban all production of it. This was contrary to the interests of the British traders dealing in opium and as a result the British sent in the gunboats to reverse the



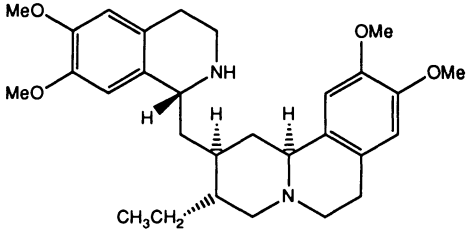
R = R' = H Morphine
R = Me R' = H Codeine
R = R' = Ac Diamorphine (Heroin)



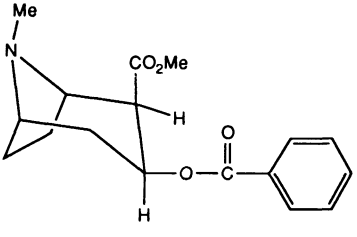
Quinine



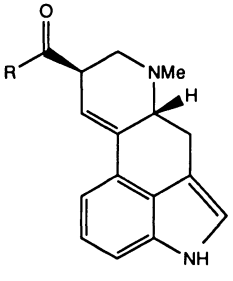
Strychnine



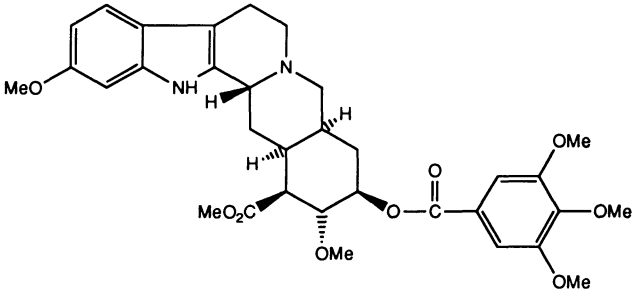
Emetine



Cocaine



R = OH Lysergic Acid
R = NEt_2 LSD



Reserpine
(Tranquilliser)

Fig. 12.1 Examples of well-known alkaloids.

Chinese decision—one of the least savoury aspects of an otherwise relatively benevolent British Empire.

Opium contains a complex mixture of almost twenty-five alkaloids. The principle alkaloid in the mixture, and the one responsible for analgesic activity, is morphine, named after the Roman god of sleep—Morpheus. Although pure morphine was isolated in 1803, it was not until 1833 that chemists at Macfarlane & Co. (now Macfarlane–Smith) in Edinburgh were able to isolate and purify it on a commercial scale. Although the functional groups on morphine had been identified by 1881, it took many more years to establish the structure of morphine and it was not until 1925 that Sir Robert Robinson solved the puzzle. Another twenty-seven years were to pass before a full synthesis of morphine was achieved in 1952.

Nevertheless, long before the structure of morphine was realized, its analgesic properties were recognized and applied to medicine. Since morphine was in the pure form, it was far more effective than crude opium as an analgesic. But there was also a price to be paid—the increased risks of addiction, tolerance, and respiratory depression.

At this stage, it is worth pointing out that all drugs have side-effects of one sort or another. This is usually due to the drug not being specific enough in its action and interacting with receptors other than the one which is of interest. One reason for drug development is to try and eliminate the side-effects without losing the useful activity. Therefore, the medicinal chemist has to try and modify the structure of the original drug molecule in order to make it more specific for the target receptor. Admittedly, this has often been a case of trial and error in the past, but there are various strategies which can be employed (see Chapter 7).

The development of narcotic analgesics is a good example of the traditional approach to medicinal chemistry and provides good examples of the various strategies which can be employed in drug development. We can identify several stages:

Stage 1. Recognition that a natural plant or herb (opium from the poppy) has a pharmacological action.
Stage 2. Extraction and identification of the active principle (morphine).
Stage 3. Synthetic studies (full synthesis and partial synthesis).
Stage 4. Structure–activity relationships—the synthesis of analogues to see which parts of the molecule are important to biological activity.
Stage 5. Drug development—the synthesis of analogues to try and improve activity or reduce side-effects.
Stage 6. Theories on the analgesic receptors. Synthesis of analogues to test theories.

Stages 5 and 6 are the most challenging and rewarding parts of the procedure as far as the medicinal chemist is concerned, since the possibility exists of improving on what Nature has provided. In this way, the chemist hopes to gain a better understanding of the biological process involved, which in turn suggests further possibilities for new drugs.

12.2 *Morphine*

12.2.1 Structure and properties

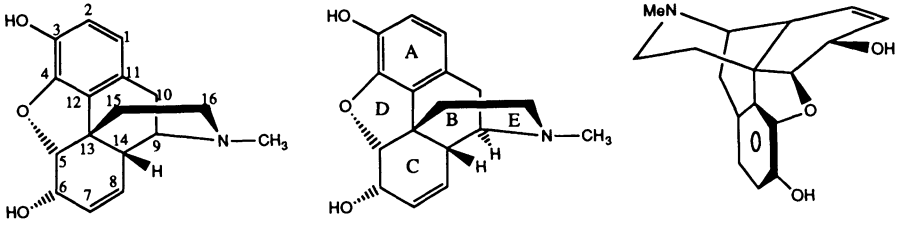


Fig. 12.2 Structure of morphine.

The active principle of opium is morphine (Fig. 12.2) and this compound is still one of the most effective painkillers available to medicine. It is especially good for treating dull, constant pain rather than sharp, periodic pain. It acts in the brain and appears to work by elevating the pain threshold, thus decreasing the brain's awareness of pain. Unfortunately, it has a large number of side-effects which include the following:

- depression of the respiratory centre
- constipation
- excitation
- euphoria
- nausea
- pupil constriction
- tolerance
- dependence

Some side-effects are not particularly serious. Some, in fact, can be advantageous. Euphoria, for example, is a useful side-effect when treating pain in terminally ill patients. Other side-effects, such as constipation, are uncomfortable but can give clues as to other possible uses for opiate-like structures. For example, opiate structures are widely used in cough medicines and the treatment of diarrhoea.

The dangerous side-effects of morphine are those of tolerance and dependence, allied with the effects morphine can have on breathing. In fact, the most common cause of death from a morphine overdose is by suffocation. Tolerance and dependence in the one drug are particularly dangerous and lead to severe withdrawal symptoms when the drug is no longer taken.

Withdrawal symptoms associated with morphine include anorexia, weight loss, pupil dilation, chills, excessive sweating, abdominal cramps, muscle spasms, hyper-irritability, lacrimation, tremor, increased heart rate, and increased blood pressure. No wonder addicts find it hard to kick the habit!

The isolation and structural identification of morphine mark the first two stages of our story and have already been described. The molecule contains five rings labelled A–E and has a pronounced T shape. It is basic because of the tertiary amino group, but it also contains a phenolic group, an alcohol group, an aromatic ring, an ether bridge, and a double bond. The next stage in the procedure is to find out which of these functional groups is essential to the analgesic activity.

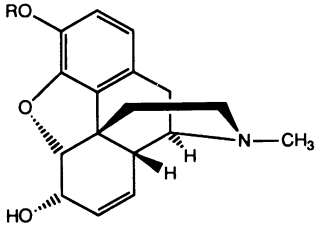
12.2.2 Structure–activity relationships

The story of how morphine's secrets were uncovered is presented here in a logical step-by-step fashion. However, in reality this was not how the problem was tackled at the time. Different compounds were made in a random fashion depending on the ease of synthesis, and the logical pattern followed on from the results obtained. By presenting the development of morphine in the following manner, we are distorting history, but we do get a better idea of the general strategies and the logical approach to drug development as a whole.

The first and easiest morphine analogues which can be made are those involving peripheral modifications of the molecule (that is, changes which do not affect the basic skeleton of the molecule). In this approach, we are looking at the different functional groups and discovering whether they are needed or not.

We now look at each of these functional groups in turn.

The phenolic OH



R = Me CODEINE
R = Et ETHYLMORPHINE
R = Acetyl 3-ACETYLMORPHINE
} Analgesic Activity ↓

Fig. 12.3

Codeine (Fig. 12.3) is the methyl ether of morphine and is also present in opium. It is used for treating moderate pain, coughs, and diarrhoea.

By methylating the phenolic OH, the analgesic activity drops drastically and codeine is only 0.1 per cent as active as morphine. This drop in activity is observed in other analogues containing a masked phenolic group. Clearly, a free phenolic group is crucial for analgesic activity.

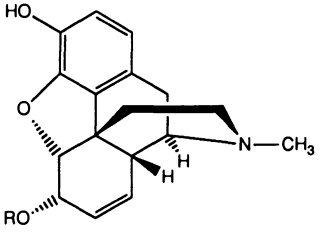
However, the above result refers to isolated receptors in laboratory experiments. If codeine is administered to patients, its analgesic effect is 20 per cent that of morphine—much better than expected. Why is this so?

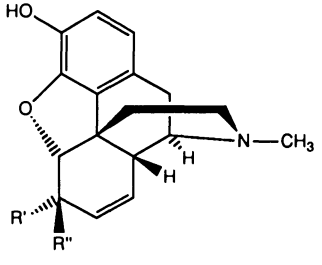
The answer lies in the fact that codeine can be metabolized in the liver to give morphine. The methyl ether is removed to give the free phenolic group. Thus, codeine can be viewed as a prodrug for morphine. Further evidence supporting this is provided by the fact that codeine has no analgesic effect at all if it is injected directly into the brain. By doing this, codeine is injected directly into the CNS and does not pass through the liver. As a result, demethylation does not take place.

This example shows the problems that the medicinal chemist can face in testing drugs. The manner in which the drugs are tested can be just as important as making the drug in the first place.

In all the following examples, the test procedures were carried out on animals or humans and so it must be remembered that there are several possible ways in which a change of activity could have resulted.

The 6-alcohol





R		Analgesia wrt morphine
Me	Heterocodeine	5x
Et	6-Ethylmorphine	greater
Acetyl	6-Acetylmorphine	4x

R'	R"	Analgesia wrt morphine
H	OH	Increased
H	H	or
Ketone	Ketone	similar

Fig. 12.4 Effect of loss of alcohol group on analgesic activity.

The results in Fig. 12.4 show that masking or the complete loss of the alcohol group does not decrease analgesic activity and, in fact, often has the opposite effect. Again, it has to be emphasized that the testing of analgesics has generally been done *in vivo* and that there are many ways in which improved activity can be achieved.

In these examples, the improvement in activity is due to the pharmacodynamic properties of these drugs rather than their affinity for the analgesic receptor. In other words, it reflects how much of the drug can reach the receptor rather than how well it binds to it.

There are a number of factors which can be responsible for affecting how much of a drug reaches its target. For example, the active compound might be metabolized to an inactive compound before it reaches the receptor. Alternatively, it might be distributed more efficiently to one part of the body than another.

In this case, the morphine analogues shown are able to reach the analgesic receptor far more efficiently than morphine itself. This is because the analgesic receptors are located in the brain and in order to reach the brain, the drugs have to cross a barrier called the blood–brain barrier. The capillaries which supply the brain are lined by a series of fatty membranes which overlap more closely than in any other part of the body. In order to enter the brain, drugs have to negotiate this barrier. Since the barrier is fatty, highly polar compounds are prevented from crossing. Thus, the more polar groups a molecule has, the more difficulty it has in reaching the brain. Morphine has three polar groups (phenol, alcohol, and an amine), whereas the analogues above have either lost the polar alcohol group or have it masked by an alkyl or acyl group. They therefore enter the brain more easily and accumulate at the receptor sites in greater concentrations; hence, the better analgesic activity.

It is interesting to compare the activities of morphine, 6-acetylmorphine, and diamorphine (heroin) (Fig. 12.5). The most active (and the most dangerous) compound of the three is 6-acetylmorphine. It is four times more active than morphine. Heroin is also more active than morphine by a factor of two, but less active than 6-acetylmorphine. How do we explain this?

6-Acetylmorphine, as we have seen already, is less polar than morphine and will enter the brain more quickly and in greater concentrations. The phenolic group is free and therefore it will interact immediately with the analgesic receptors.

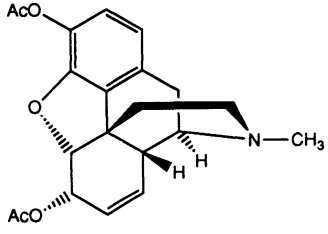


Fig. 12.5 Diamorphine (heroin).

Heroin has two polar groups which are masked and is therefore the most efficient compound of the three to cross the blood–brain barrier. However, before it can act at the receptor, the acetyl group on the phenolic group has to be removed by esterases in the brain. Therefore, it is more powerful than morphine because it enters the brain more easily, but it is less powerful than 6-acetylmorphine because the 3-acetyl group has to be removed before it can act.

Heroin and 6-acetylmorphine are both more potent analgesics than morphine. Unfortunately, they also have greater side-effects and have severe tolerance and dependence characteristics. Heroin is still used to treat terminally ill patients, such as those dying of cancer, but 6-acetylmorphine is so dangerous that its synthesis is banned in many countries.

To conclude, the 6-hydroxyl group is not required for analgesic activity and its removal can be beneficial to analgesic activity.

The double bond at 7–8

Several analogues including dihydromorphine (Fig. 12.6) have shown that the double bond is not necessary for analgesic activity.

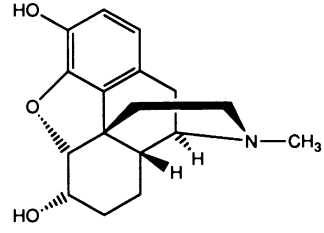
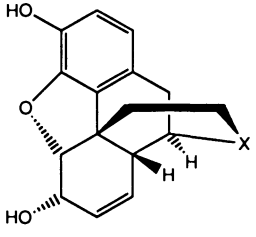


Fig. 12.6 Dihydromorphine.

The *N*-methyl group

The *N*-oxide and the *N*-methyl quaternary salts of morphine are both inactive, which might suggest that the introduction of charge destroys analgesic activity (Fig. 12.7). However, we have to remember that these experiments were done on animals and it is hardly surprising that no analgesia is observed, since a charged molecule has very little chance of crossing the blood–brain barrier. If these same compounds are injected directly into the brain, a totally different result is obtained and both these compounds are found to have similar analgesic activity to morphine. This fact, allied with the fact that neither compound can lose its charge, shows that the nitrogen atom of morphine is ionized when it binds to the receptor.



X		Analgesic Activity wrt morphine
NH	Normorphine	25%
	N-Oxide	0%
	Quaternary salt	0%

Fig. 12.7 Effect of introduction of charge on analgesic activity.

The replacement of the *N*-methyl group with a proton reduces activity but does not eliminate it. The secondary NH group is more polar than the tertiary *N*-methyl group and therefore finds it more difficult to cross the blood–brain barrier, leading to a drop in activity. The fact that significant activity is retained despite this shows that the methyl substituent is not essential to activity.

However, the nitrogen itself is crucial. If it is removed completely, all analgesic

activity is lost. To conclude, the nitrogen atom is essential to analgesic activity and interacts with the analgesic receptor in the ionized form.

The aromatic ring

The aromatic ring is essential. Compounds lacking it show no analgesic activity.

The ether bridge

As we shall see later, the ether bridge is not required for analgesic activity.

Stereochemistry

At this stage, it is worth making some observations on stereochemistry. Morphine is an asymmetric molecule containing several chiral centres, and exists naturally as a single enantiomer. When morphine was first synthesized, it was made as a racemic mixture of the naturally occurring enantiomer plus its mirror image. These were separated and the unnatural mirror image was tested for analgesic activity. It turned out to have no activity whatsoever.

This is not particularly surprising if we consider the interactions which must take place between morphine and its receptor. We have identified that there are at least three important interactions involving the phenol, the aromatic ring and the amine on

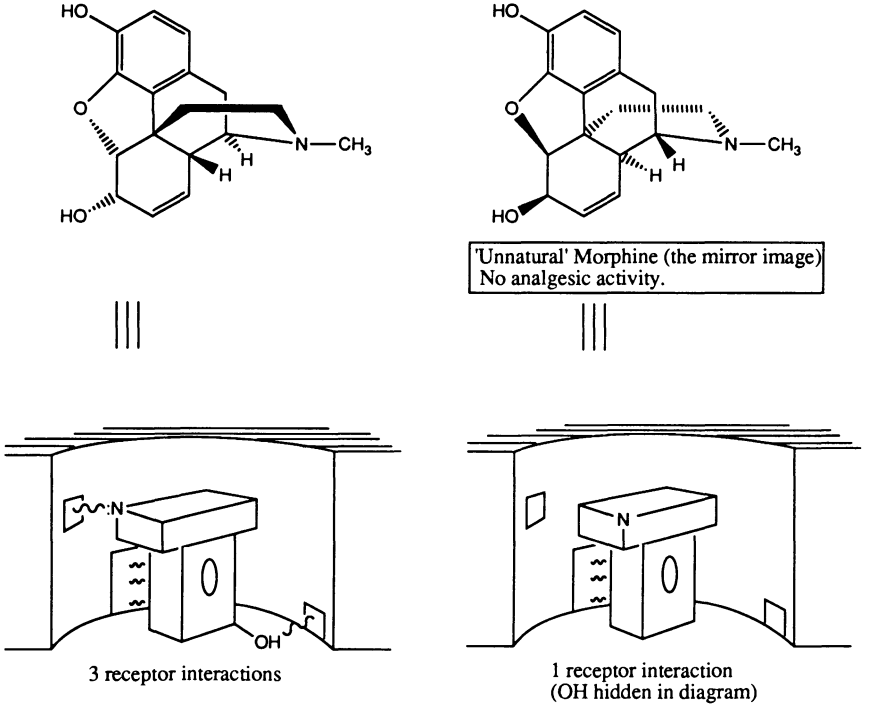


Fig. 12.8 Morphine and 'unnatural' morphine.

morphine. Let us consider a diagrammatic representation of morphine as a T-shaped block with the three groups marked as shown in Fig. 12.8. The receptor has complementary binding groups placed in such a way that they can interact with all three groups. If we now consider the mirror image of morphine, then we can see that it can interact with only one binding site at any one time.

Epimerization of a single chiral centre such as the 14-position (Fig. 12.9) is not beneficial either, since changing the stereochemistry at even one chiral centre can result in a drastic change of shape, making it impossible for the molecule to bind to the analgesic receptors.

To sum up, the important functional groups for analgesic activity in morphine are shown in Fig. 12.10.

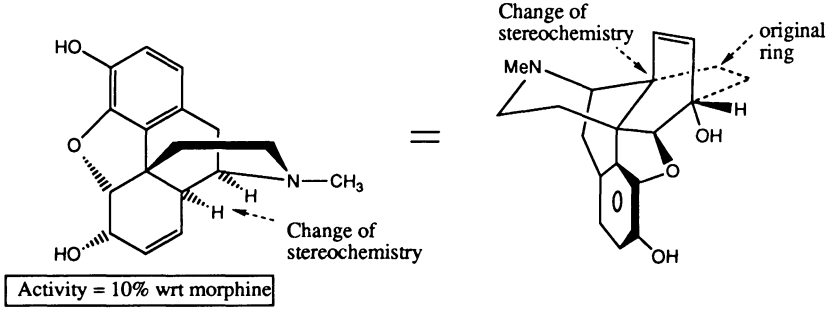


Fig. 12.9 Epimerization of a single chiral centre.

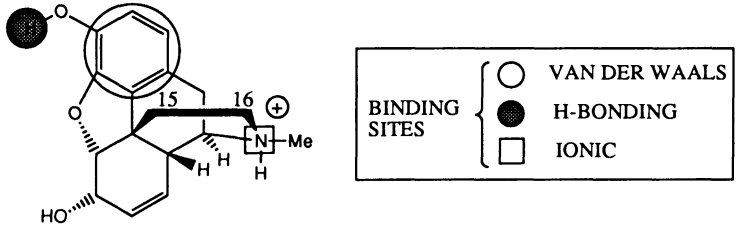


Fig. 12.10 Important functional groups for analgesic activity in morphine.

12.3 *Development of morphine analogues*

We now move on to consider the development of morphine analogues. As mentioned in Chapter 7, there are several strategies used in drug development.

We shall consider the following strategies in the development of morphine analogues.

- variation of substituents
- drug extension
- simplification
- rigidification

12.3.1 Variation of substituents

A series of alkyl chains on the phenolic group give compounds which are inactive or poorly active. We have already identified that the phenol group must be free for analgesic activity.

The removal of the *N*-methyl group to give normorphine allows a series of alkyl chains to be built on the basic centre. These results are discussed under drug extension since the results obtained are more relevant under that heading.

12.3.2 Drug extension

Drug extension is a strategy by which the molecule is 'extended' by the addition of extra 'binding groups'. The reasoning behind such a tactic is to probe for further binding sites which might be available on the receptor surface and which might improve the interaction between the drug and the receptor (Fig. 12.11).

This is a reasonable assumption since it is highly unlikely that a compound such as morphine (which is produced in a plant) would be the perfect binding substrate for a receptor in the human brain

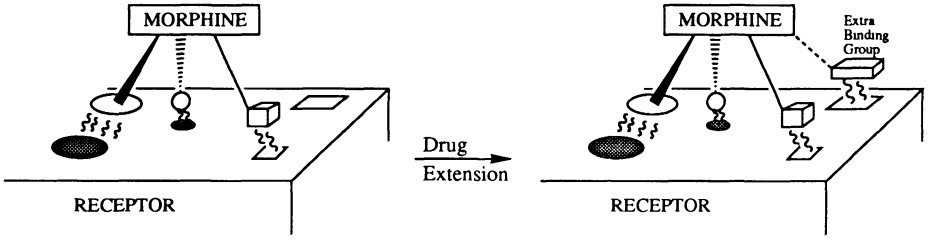


Fig. 12.11 Drug extension of morphine.

Many analogues of morphine have been made with extra functional groups attached. These have rarely shown any improvement. However, there are two exceptions. The introduction of a hydroxyl group at position 14 has been particularly useful (Fig. 12.12). This might be taken to suggest that there is a possible hydrogen bond interaction taking place between the 14-OH group and a suitable amino acid residue on the receptor. However, an alternative explanation is provided in Section 12.5.

The easiest position to add substituents (and the most advantageous) has been the nitrogen atom. The synthesis is easily achieved by removing the *N*-methyl group from morphine to give normorphine, then alkylating the amino group with an alkyl halide. Removal of the *N*-methyl group was originally achieved by a von Braun

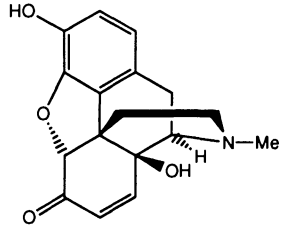


Fig. 12.12 Oxymorphine (2.5 × activity of morphine).

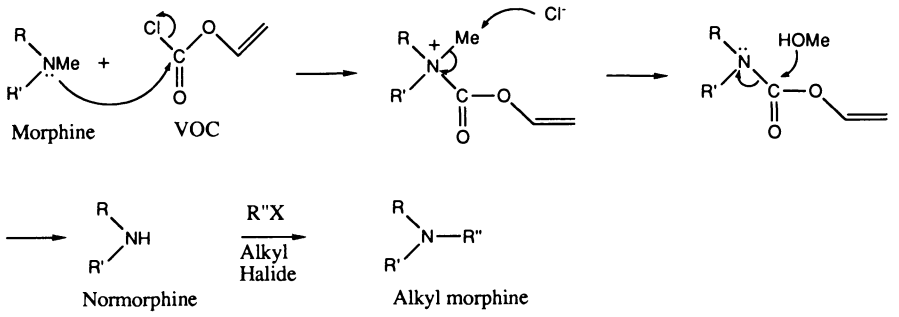


Fig. 12.13 Demethylation and alkylation of the basic centre.

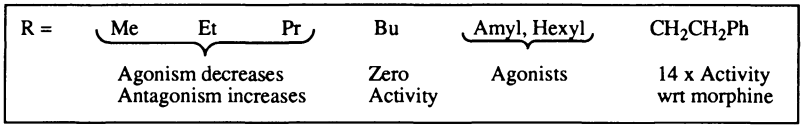


Fig. 12.14 Change in activity with respect to alkyl group size.

degradation with cyanogen bromide, but is now more conveniently carried out using a chloroformate reagent such as vinyloxycarbonyl chloride (Fig. 12.13). The alkylation step can sometimes be profitably replaced by a two-step process involving an acylation to give an amide, followed by reduction.

The results obtained from the alkylation studies are quite dramatic. As the alkyl group is increased in size from a methyl to a butyl group, the activity drops to zero (Fig. 12.14). However, with a larger group such as an amyl or a hexyl group, activity recovers slightly. None of this is particularly exciting, but when a phenethyl group is attached the activity increases 14 fold—a strong indication that a hydrophobic binding site has been located which interacts favourably with the new aromatic ring (Fig. 12.15).

To conclude, the size and nature of the group on the nitrogen is important to the activity spectrum. Drug extension can lead to better binding by making use of additional binding interactions.

Before leaving this subject, it is worth describing another series of important results arising from varying substituents on the nitrogen atom. Spectacular results were obtained when an allyl group or a cyclopropylmethylene group were attached (Fig. 12.16).

No increase in analgesic activity was observed

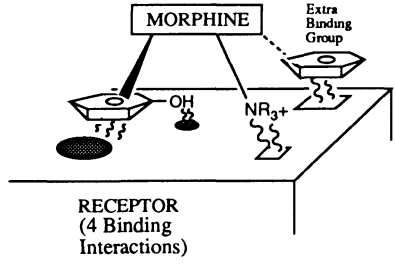


Fig. 12.15 Indication of fourth binding site.

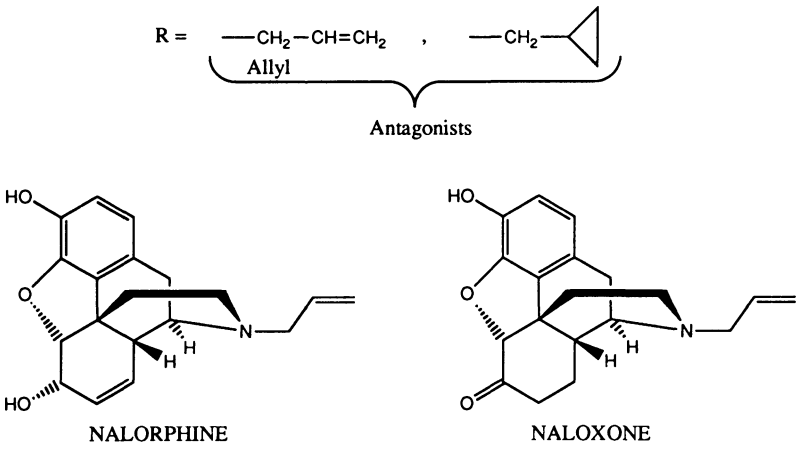


Fig. 12.16

and in fact the results were quite the opposite. Naloxone, for example, has no analgesic activity at all, whilst nalorphine retains only weak analgesic activity. However, the important feature about these molecules is that they act as antagonists to morphine. They do this by binding to the analgesic receptors without 'switching them on'. Once they have bound to the receptors, they block morphine from binding. As a result, morphine can no longer act as an analgesic. One might be hard pushed to see an advantage in this and with good reason. If we are just considering analgesia, there is none. However, the fact that morphine is blocked from all its receptors means that none of its side-effects are produced either, and it is the blocking of these effects which make antagonists extremely useful.

In particular, accident victims have sometimes been given an overdose of morphine. If this is not treated, then the casualty may die of suffocation.

By administering nalorphine, the antagonist displaces morphine from the receptor and binds more strongly, thus preventing morphine from continuing its action.

There is, however, a far more important observation arising from the biological results of these antagonists. For many years, chemists had been trying to find a morphine analogue with analgesic properties, but without the depressant effects on breathing, or the withdrawal symptoms. There had been so little success that many workers believed that the two properties were directly related, perhaps through the same receptor. The fact that the antagonist naloxone blocked morphine analgesia and side-effects at the same time did nothing to change that view.

However, the properties of nalorphine offered a glimmer of hope. Nalorphine is a strong antagonist and blocks morphine from its receptors. Therefore, no analgesic activity should be observed. However, a very weak analgesic activity *is* observed and what is more, this analgesia appears to be free of the undesired side-effects. This was the first sign that a non-addictive, safe analgesic might be possible.

But how can this be? How can a compound be an antagonist of morphine but also act as an agonist and produce analgesia. If it is acting as an agonist, why is the activity so weak and why is it free of the side-effects?

As we shall see later, there is not one single type of analgesic receptor, but several. Multiple receptors are common. We have already seen in Chapter 11 that there are two types of acetylcholine receptor—the nicotinic and muscarinic.

In the same way, there are at least three types of analgesic receptor. The differences between them are slight such that morphine cannot distinguish between them and activates them all, but in theory it should be possible to find compounds which would be selective for one type of analgesic receptor over another. However, this is not the way that nalorphine works.

Nalorphine binds to all three types of analgesic receptor and therefore blocks morphine from all three. Nalorphine itself is unable to switch on two of the receptors and is therefore a true antagonist at these receptors. However, at the third type of receptor, nalorphine is acting as a weak or partial agonist (see Section 5.8.). In other words, it has activated the receptor, but only weakly. We could imagine how this might occur if the third receptor is controlling something like an ion channel (Fig. 12.17).

Morphine is a strong agonist and interacts strongly with this receptor leading to a change in receptor conformation which fully opens the ion channel. Ions flow in or out of the cell, resulting in the activation or deactivation of enzymes. Naloxone is a pure antagonist. It binds strongly, but does not produce a change in the receptor conformation. Therefore, the ion channel remains closed. Nalorphine binds to the third receptor and changes the tertiary structure of the receptor very slightly, leading to a slight opening of the ion channel. It is therefore a weak agonist at this receptor, but it is also an antagonist since it blocks morphine from fully 'switching on' the receptor.

The results observed with nalorphine show that activation of this third type of analgesic receptor leads to analgesia without the undesirable side-effects associated with the other two analgesic receptors.

Unfortunately, nalorphine has hallucinogenic side-effects resulting from the activation of a non-analgesic receptor, and is therefore unsuitable as an analgesic, but for the first time a certain amount of analgesia had been obtained without the side-effects of respiratory depression and tolerance.

12.3.3 Simplification or drug dissection

We turn now to more drastic alterations of the morphine structure and ask whether the complete carbon skeleton is really necessary. After all, if we could simplify the molecule, it would be easier to make in the laboratory. This in turn would allow the chemist to make analogues much more easily, and any useful compounds could be made more efficiently and cheaply.

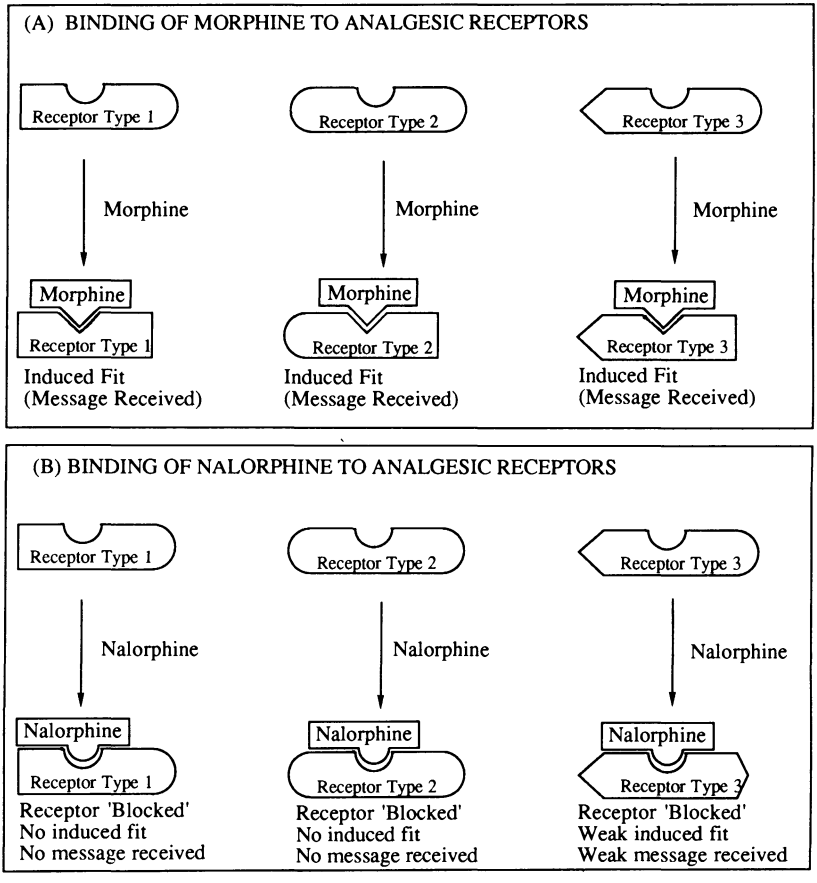


Fig. 12.17 Action of morphine and nalorphine at analgesic receptors.

There are five rings present in the structure of morphine (Fig. 12.18) and analogues were made to see which rings could be removed.

Removing ring E

Removing ring E leads to a complete loss of activity. This result emphasizes the importance of the basic nitrogen to analgesic activity.

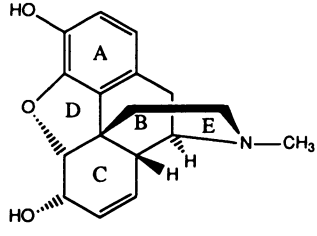
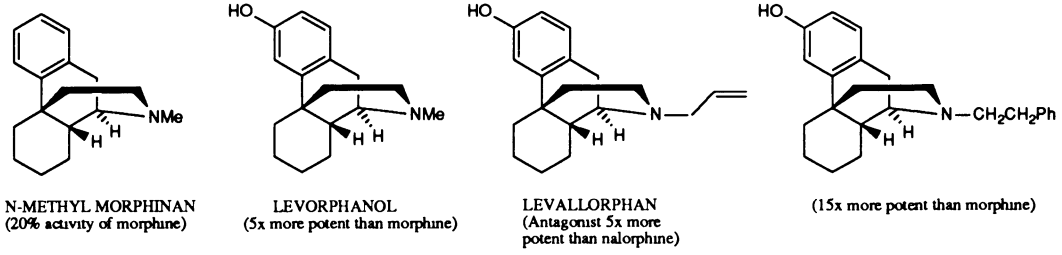


Fig. 12.18 Structure of morphine.

Removing ring D

Removing the oxygen bridge gives a series of compounds called the morphinans which have useful analgesic activity. This demonstrate that the oxygen bridge is not essential. Examples are shown in Fig. 12.19.



N-METHYL MORPHINAN (20% activity of morphine)

LEVORPHANOL (5x more potent than morphine)

LEVALLORPHAN (Antagonist 5x more potent than nalorphine)

(15x more potent than morphine)

Fig. 12.19 Examples of morphinans.

N-Methyl morphinan was the first such compound tested and is only 20 per cent as active as morphine, but since the phenolic group is missing, this is not surprising. The more relevant levorphanol structure is five times more active than morphine and, although side-effects are also increased, levorphanol has a massive advantage over morphine in that it can be taken orally and lasts much longer in the body. This is because levorphanol is not metabolized in the liver to the same extent as morphine.

As might be expected, the mirror image of levorphanol (dextrorphan) has insignificant analgesic activity.

The same strategy of drug extension already described for the morphine structures was also tried on the morphinans with similar results. For example, adding an allyl substituent on the nitrogen gives antagonists. Adding a phenethyl group to the nitrogen greatly increases potency. Adding a 14-OH group also increases activity.

Conclusions:

- Morphinans are more potent and longer acting than their morphine counterparts, but they also have higher toxicity and comparable dependence characteristics.
- The modifications carried out on morphine, when carried out on the morphinans, lead to the same biological results. This implies that both types of molecule are reacting with the same receptors in the same way.
- The morphinans are easier to synthesize since they are simpler molecules.

Removing rings C and D

Opening both rings C and D gives an interesting group of compounds called the benzomorphans (Fig. 12.20) which are found to retain analgesic activity. One of the simplest of these structures is metazocine which has the same analgesic activity as

morphine. Notice that the two methyl groups in metazocine are *cis* with respect to each other and represent the 'stumps' of the C ring.

If the same type of chemical modifications are carried out on the benzomorphans as were described for the morphinans and morphine, then the same biological effects are observed. This suggests that the benzomorphans interact with the same receptors as the morphinans and morphine analogues. For example, replacing the *N*-methyl group of metazocine with a phenethyl group gives phenazocine which is four times more active than morphine and the first compound to have a useful level of analgesia without dependence properties.

Further developments led to pentazocine (Fig. 12.21) which has proved to be a useful long-term analgesic with a very low risk of addiction. A newer compound (bremazocine) has a longer duration, is 200 times the activity of morphine, appears to have no addictive properties, and does not depress breathing.

These compounds appear to be similar in their action to nalorphine in that they act as antagonists at two of the three types of analgesic receptors, but act as an agonist at the third. The big difference between nalorphine and compounds like pentazocine is that the latter are far stronger agonists, resulting in a more useful level of analgesia.

Unfortunately, many of these compounds have hallucinogenic side-effects due to interactions with a non-analgesic receptor.

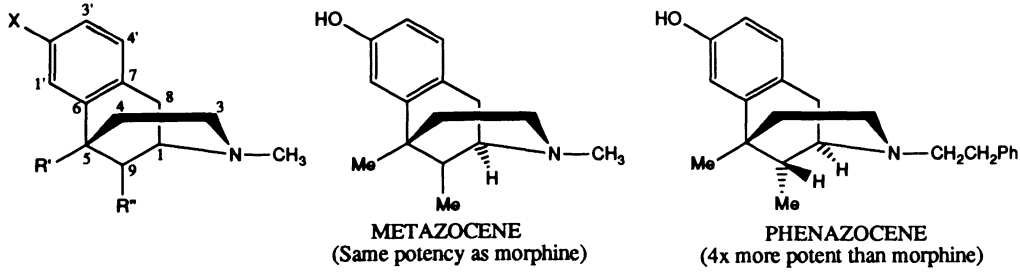


Fig. 12.20 Benzomorphans.

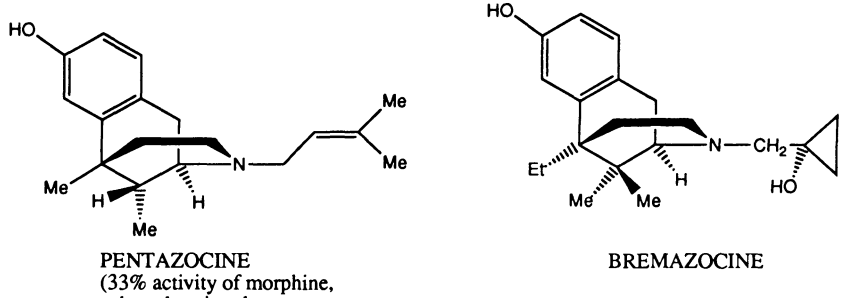


Fig. 12.21 Benzomorphans with low rates of dependency.

We shall come back to the interaction of benzomorphans with analgesic receptors later. For the moment, we can make the following conclusions about benzomorphans.

- Rings C and D are not essential to analgesic activity.
- Analgesia and addiction are not necessarily coexistent.
- 6,7-Benzomorphans are clinically useful compounds with reasonable analgesic activity, less addictive liability, and less tolerance.
- Benzomorphans are simpler to synthesize.

Removing rings B, C, and D

Removing rings B, C, and D gives a series of compounds known as 4-phenylpiperidines. The analgesic activity of these compounds was discovered by chance in the 1940s when chemists were studying analogues of cocaine for antispasmodic properties. Their structural relationship to morphine was only identified when they were found to be analgesics, and is evident if the structure is drawn as shown in Fig. 12.22. Activity can be increased sixfold by introducing the phenolic group and altering the ester to a ketone to give ketobemidone.

Meperidine (pethidine) is not as strong an analgesic as morphine and also shares the same undesirable side-effects. However, it has a rapid onset and a shorter duration and as a result has been used as an analgesic for difficult childbirths. The rapid onset and short duration of meperidine mean that there is less chance of it depressing the baby's breathing.

The piperidines are more easily synthesized than any of the above groups and a large number of analogues have been studied. There is some doubt as to whether they act in the same way as morphine at analgesic receptors since some of the chemical adaptations we have already described do not lead to comparable biological results. For example, adding allyl or cyclopropyl groups does not give antagonists. The replacement of the methyl group of meperidine with a cinnamic acid residue increases the activity by 30 times, whereas putting the same group on morphine eliminates activity (Fig. 12.23).

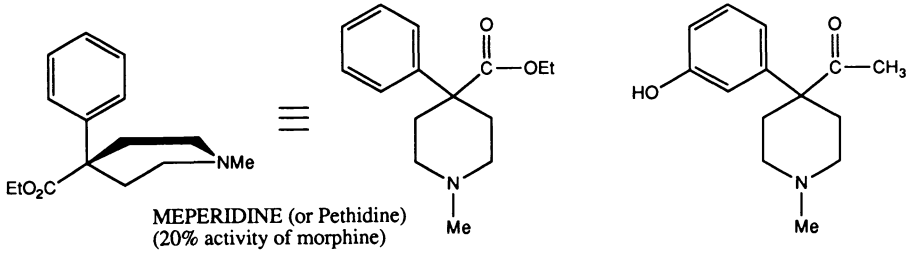
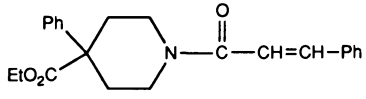
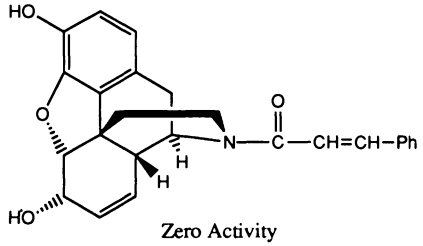


Fig. 12.22 4-phenyl piperidines.



30x more potent than pethidine



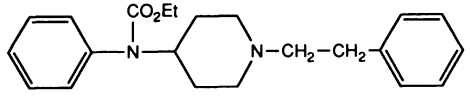
Zero Activity

Fig. 12.23 Effect of addition of a cinnamic acid residue on meperidine and morphine.

These results might have something to do with the fact that the piperidines are far more flexible molecules than the previous structures and are thus more likely to interact with receptors in different ways.

One of the most successful piperidine derivatives is fentanyl (Fig. 12.24) which is up to 100 times more active than morphine. The drug lacks a phenolic group, but is very lipophilic. As a result, it can cross the blood–brain barrier efficiently.

Fig. 12.24 Fentanyl (no 2-C bridge).



Conclusions:

- Rings C, D, and E are not essential for analgesic activity.
- Piperidines retain side-effects such as addiction and depression of the respiratory centre.
- Piperidine analgesics are faster acting and have shorter duration.
- The quaternary centre present in piperidines is usually necessary (fentanyl is an exception).
- The aromatic ring and basic nitrogen are essential to activity, but the phenol group is not.
- Piperidine analgesics appear to interact with analgesic receptors in a different manner to previous groups.

Removing rings B, C, D, and E

The analgesic methadone (Fig. 12.25) was discovered in Germany during the Second World War and has proved to be a useful agent comparable in activity to morphine. Unfortunately, methadone retains morphine-like side-effects. However, it is orally active and has less severe emetic and constipation effects. Side-effects such as sedation, euphoria, and withdrawal are also less severe and therefore the compound has been given to drug addicts as a substitute for morphine (or heroin) in order to wean them

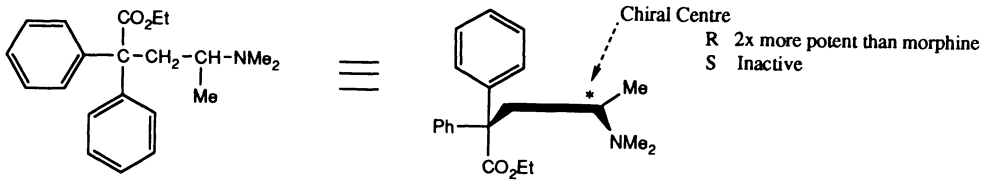


Fig. 12.25 Methadone.

off these drugs. This is not a complete cure since it merely swaps an addiction to heroin for an addiction to methadone. However, this is considered less dangerous.

The molecule has a single chiral centre and when the molecule is drawn in the same manner as morphine, we would expect the *R* enantiomer to be the more active enantiomer. This proves to be the case with the *R* enantiomer being twice as powerful as morphine, whereas the *S* enantiomer is inactive. This is quite a dramatic difference. Since the *R* and *S* enantiomers have identical physical properties and lipid solubility, they should both reach the receptor site to the same extent, and so the difference in activity is most probably due to receptor–substrate interactions.

Many analogues of methadone have been synthesized, but with little improvement over the parent drug.

12.3.4 Rigidification

Up till now, we have considered minor adjustments of functional groups on the periphery of the morphine skeleton or drastic simplification of the morphine skeleton.

A completely different strategy is to make the molecule more complicated or more rigid. This strategy is usually employed in an attempt to remove the side-effects of a drug or to increase activity.

It is usually assumed that the side-effects of a drug are due to interactions with additional receptors other than the one we are interested in. These interactions are probably due to the molecule taking up different conformations or shapes. If we make the molecule more rigid so that it takes up fewer conformations, we might eliminate the conformations which are recognized by undesirable receptors, and thus restrict the molecule to the specific conformation which fits the desired receptor. In this way, we would hope to eliminate such side-effects as dependence and respiratory depression. We might also expect increased activity since the molecule is more likely to be in the correct conformation to interact with the receptor.

The best example of this tactic in the analgesic field is provided by a group of compounds known as the oripavines. These structures often show remarkably high activity.

The oripavines are made from an alkaloid which we have not described so far—thebaine (Fig. 12.26). Thebaine can be extracted from opium along with codeine and

morphine and is very similar in structure to both these compounds. However, unlike morphine and codeine, thebaine has no analgesic activity. There is a diene group present in ring C of thebaine and when thebaine is reacted with methyl vinyl ketone, a Diels–Alder reaction takes place to give an extra ring and increased rigidity to the structure (Fig. 12.26).

A comparison with morphine shows that the extra ring sticks out from what used to be the 'crossbar' of the T-shaped structure (Fig. 12.27).

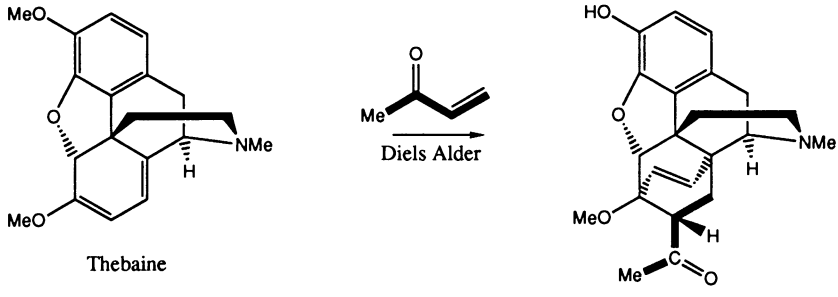


Fig. 12.26 Formation of oripavines.

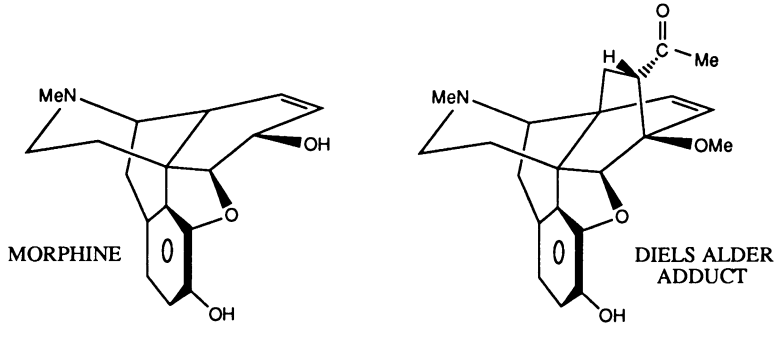


Fig. 12.27 Comparison of morphine and oripavine.

Since a ketone group has been introduced, it is now possible to try the strategy of drug extension, this time by adding various groups to the ketone via a Grignard reaction (Fig. 12.28).

It is noteworthy that the Grignard reaction is stereospecific. The Grignard reagent complexes to both the 6-methoxy group and the ketone, and is then delivered to the less-hindered face of the ketone to give an asymmetric centre (Fig. 12.29).

By varying the groups added by the Grignard reaction, some remarkably powerful compounds have been obtained. Etorphine (Fig. 12.30), for example, is 10 000 times more potent than morphine. This is a combination of the fact that it is a very hydrophobic molecule and can cross the blood–brain barrier 300 times more easily

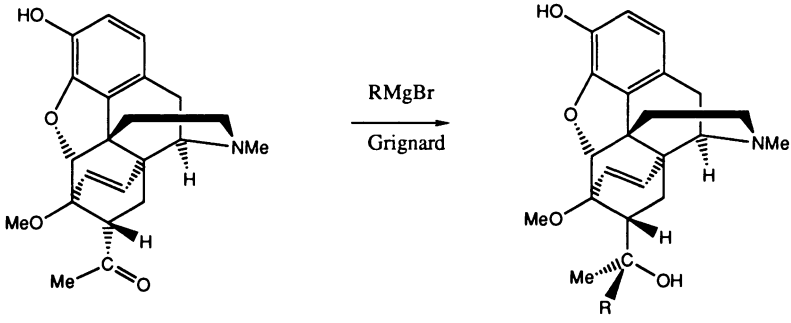


Fig. 12.28 Drug extension.

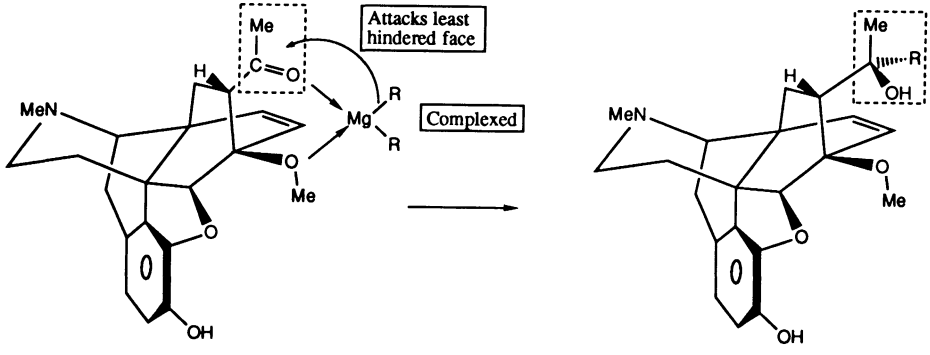


Fig. 12.29 Grignard reaction leads to an asymmetric centre.

than morphine, as well as the fact that it has 20 times more affinity for the analgesic receptor site due to better binding interactions.

At slightly higher doses than those required for analgesia, it can act as a 'knock-out' drug or sedative. The compound has a considerable margin of safety and is used to immobilize large animals such as elephants. Since the compound is so active, only very small doses are required and these can be dissolved in such small volumes (1 ml) that they can be placed in crossbow darts and fired into the hide of the animal.

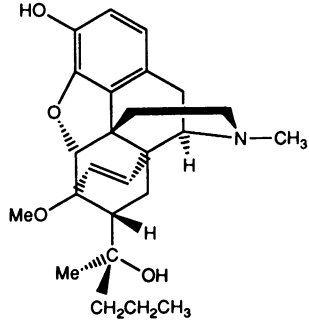


Fig. 12.30 Etorphine.

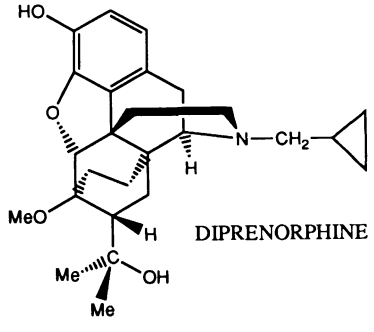
The addition of lipophilic groups (R) (Fig. 12.29) is found to improve activity dramatically, indicating the presence of a hydrophobic binding region close by on the receptor.[1] The group best able to interact with this region is a phenethyl substituent and the product containing this group is even more active than etorphine.

[1] It is believed that the phenylalanine aromatic ring on enkephalins (see later) interacts with this same binding site.

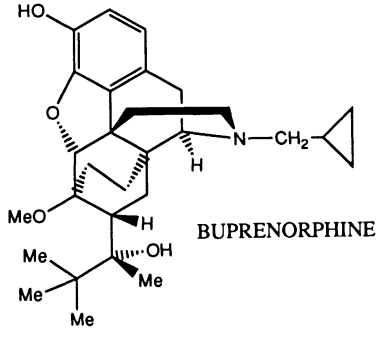
As one might imagine, these highly active compounds have to be handled very carefully in the laboratory.

Because of their rigid structures, these compounds are highly selective agents for the analgesic receptors. Unfortunately, the increased analgesic activity is also accompanied by unacceptable side-effects. It was therefore decided to see if putting substituents on the nitrogen, such as an allyl or cyclopropyl group, would give antagonists as found in the morphine, morphinan, and benzomorphan series of compounds. If so, it might be possible to obtain an oripavine equivalent of a pentazocine or a nalorphine—an antagonist with some agonist activity and with reduced side-effects.

Putting on a cyclopropyl group gives a very powerful antagonist called diprenorphine (Fig. 12.31), which is 100 times more potent than nalorphine and can be used to reverse the immobilizing effects of etorphine (see above). Diprenorphine has no analgesic activity.



DIPRENORPHINE



BUPRENORPHINE (1968)

Fig. 12.31

Replacing the methyl group derived from the Grignard reagent with a *t*-butyl group gives buprenorphine (Fig. 12.31) which has similar properties to drugs like nalorphine and pentazocine, in that it has analgesic activity with a very low risk of addiction. This feature appears to be related to the slow onset and removal of buprenorphine from the analgesic receptors. Since these effects are so gradual, the receptor system is not subjected to sudden changes in transmitter levels.

Buprenorphine is the most lipophilic compound in the oripavine series of compounds and therefore enters the brain very easily. Usually, such a drug would react quickly with its receptor. The fact that it does not is therefore a feature of its interaction with the receptor rather than the ease with which it can reach the receptor. It is 100 times more active than morphine as an agonist and four times more active than nalorphine as an antagonist. It is a particularly safe drug since it has very little effect on respiration and what little effect it does have actually decreases at high doses. Therefore, the risks of suffocation from a drug overdose are much smaller than with

morphine. Buprenorphine has been used in hospitals to treat patients suffering from cancer and also following surgery. Its drawbacks include side-effects such as nausea and vomiting as well as the fact that it cannot be taken orally. A further use for buprenorphine is as an alternative means to methadone for weaning addicts off heroin.

Buprenorphine binds slowly to analgesic receptors, but once it does bind, it binds very strongly. As a result, less buprenorphine is required to interact with a certain percentage of analgesic receptors than morphine.

On the other hand, buprenorphine is only a partial agonist. In other words, it is not very efficient at switching the analgesic receptor on. This means that it is unable to reach the maximum level of analgesia which can be acquired by morphine.

Overall, buprenorphine's stronger affinity for analgesic receptors outweighs its relatively weak action such that a lower dose of buprenorphine can produce analgesia, compared to morphine. However, if pain levels are high, buprenorphine is unable to counteract the pain and morphine has to be used.

Nevertheless, buprenorphine provides another example of an opiate analogue where analgesia has been separated from dangerous side-effects.

It is time to look more closely at the receptor theories relevant to the analgesics.

12.4 *Receptor theory of analgesics*

Although it has been assumed for many years that there are analgesic receptors, information about them has only been gained relatively recently (1973).

The present knowledge on the subject is that there are at least four different receptors with which morphine can interact, three of which are analgesic receptors. The initial theory on receptor binding assumed a single receptor site, but this does not invalidate many of the proposals which were made. Therefore, it is informative to look at the first theory—the Beckett–Casy hypothesis.

12.4.1 Beckett–Casy hypothesis

In this theory, it is assumed that there is a rigid receptor site and that morphine and its analogues fit into the site in a classic lock-and-key analogy.

Based on the results already described, the following features were proposed as being essential if an analgesic is to interact with its receptor.

- There must be a basic centre (nitrogen) which can be ionized at physiological pH to form a positively charged group. This group then forms an ionic bond with a comparable anionic group in the receptor. As a consequence of this, analgesics have to have a pK_a of 7.8–8.9 such that there is an approximately equal chance of the amine being ionized or un-ionized at physiological pH. This is necessary since the

analgesic has to cross the blood–brain barrier as the free base, but once across has to be ionized in order to interact with the receptor.

The pK_a values of useful analgesics all match this prediction.

- The aromatic ring in morphine has to be properly orientated with respect to the nitrogen atom to allow a van der Waals interaction with a suitable hydrophobic location on the receptor. The nature of this interaction suggests that there has to be a close spatial relationship between the aromatic ring and the surface of the receptor.
- The phenol group is probably hydrogen-bonded to a suitable residue at the receptor site.
- There might be a 'hollow' just large enough for the ethylene bridge of carbons 15 and 16 to fit. Such a fit would help to align the molecule and enhance the overall fit.

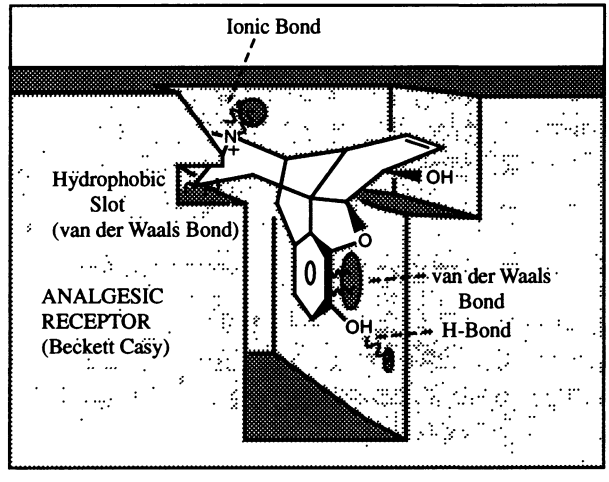


Fig. 12.32 Beckett–Casy hypothesis.

This was the first theory proposed and fitted in well with the majority of results. There can be no doubt that the aromatic ring, phenol, and the nitrogen groups are all important, but there is some doubt as to whether the ethylene bridge is important, since there are several analgesics which lack it (e.g. fentanyl).

The theory also fails to include the extra binding site which was discovered by drug extension. This fact can easily be fitted into the theory, but other anomalies exist which have already been discussed (e.g. the different results obtained for meperidine compared to morphine when a substituent such as the allyl group is attached to nitrogen).

Another anomaly was described earlier where the pethidine analogue containing a cinnamic acid residue is 30 times more active than pethidine itself, whereas the same group on morphine eliminates activity. Such results strongly suggest that a simple one-receptor theory is not applicable.

12.4.2 Multiple analgesic receptors

The previous theory tried to explain analgesic results based on a single analgesic receptor. It is now known that there are several different analgesic receptors which are associated with different types of side-effects. It is also known that several analgesics show preference for some of these receptors over others. This helps to explain the anomalies resulting from the previous Beckett–Casy hypothesis.

It is important to appreciate that the main points of the original theory still apply for each of the analgesic receptors now to be described. The important binding groups for each receptor are the phenol, the aromatic ring, and the ionized nitrogen centre. However, there are subtle differences between each receptor which can distinguish between the finer details of different analgesic molecules. As a result, some analgesics show preference for one analgesic receptor over another or interact in different ways.

There are three analgesic receptors to which the morphine molecule itself can bind and 'switch on'. These receptors have been tabbed with Greek letters.

The mu receptor (μ)

Morphine binds strongly to this receptor and produces analgesia. Receptor binding also leads to the undesired side-effects of respiratory depression, euphoria, and addiction. We can now see why it is so difficult to remove the side-effects of morphine and is analogues, since the receptor with which they bind most strongly is also inherently involved with these side-effects.

The kappa receptor (κ)

This is a different analgesic receptor to which morphine can bind and activate. However, the strength of binding is less than to the μ receptor.

The biological response is analgesia with sedation and none of the hazardous side-effects. It is this receptor which provides the best hope for the ultimate safe analgesic. The earlier results obtained from nalorphine, pentazocine, and buprenorphine can now be explained.

Nalorphine acts as an antagonist at the μ receptor, thus blocking morphine from acting there. However, it acts as a weak agonist at the κ receptor (as does morphine) and so the slight analgesia observed with nalorphine is due to the partial activation of the κ receptor. Unfortunately, nalorphine has hallucinogenic side-effects. This is caused by nalorphine also binding to a completely different, non-analgesic receptor in the brain called the sigma receptor (σ) (see Section 12.7.4.) where it acts as an agonist.

Pentazocine interacts with the μ and κ receptors in the same way, but is able to 'switch on' the κ receptor more strongly. It too suffers the drawback that it 'switches on' the σ receptor. Buprenorphine is slightly different. It binds strongly to all three analgesic receptors and acts as an antagonist at the Δ (see below) and κ receptors, but

acts as a partial agonist at the μ receptor to produce its analgesic effect. This might suggest that buprenorphine should suffer the same side-effects as morphine. The fact that it does not is related in some way to the rate at which buprenorphine interacts with the receptor. It is slow to bind, but once it has bound, it is slow to leave.

The delta receptor (Δ)

The Δ receptor is where the brain's natural painkillers (the enkephalins, Section 12.6.) interact. Morphine can also bind quite strongly to this receptor.

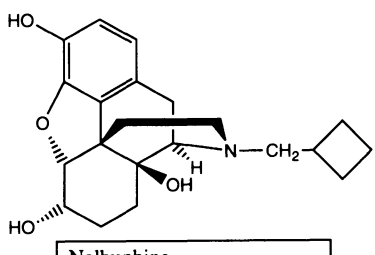
A table showing the relative activities of morphine, nalorphine, pentazocine, enkephalins, pethidine, and naloxone is shown in Fig. 12.33. A plus sign indicates the compound is acting as an agonist. A minus sign means it acts as an antagonist. A zero sign means there is no activity or minor activity.

There is now a search going on for orally active opiate structures which can act as antagonists at the μ receptor, agonists at the κ receptor, and have no activity at the σ receptor. Some success has been obtained, especially with the compounds shown in Fig. 12.34, but even these compounds still suffer from certain side-effects, or lack the desired oral activity.

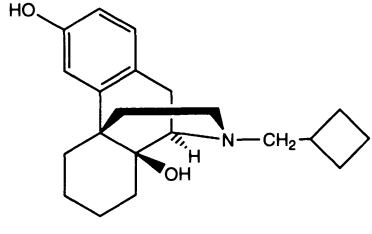
		Morphine	Nalorphine	Pentazocine	Enkephalins	Pethidine	Naloxone
Mu	Analgesia respiratory depression euphoria addiction	+++	−	−	+	+++	−−−
Kappa	Analgesia sedation	+	+	+	+	+	−
Sigma	Psychotomimetic	0	+	+	0	0	0
Delta	Analgesia	++	−	−	+++	+	−

+, Compounds acting as agonists; −, as antagonists. 0, No activity or minor activity.

Fig. 12.33 Relative activities of analgesics.



Nalbuphine
(same activity as morphine)
Low addiction Liability
No psychotomimetic activity
Not orally active



Butorphanol
(Not orally active)

Fig. 12.34 New analgesic structures.

12.5 *Agonists and antagonists*

We return now to look at a particularly interesting problem regarding the agonist/antagonist properties of morphine analogues. Why should such a small change as replacing an *N*-methyl group with an allyl group result in such a dramatic change in biological activity such that an agonist becomes an antagonist? Why should a molecule such as nalorphine act as an agonist at one analgesic receptor and an antagonist at another? How can different receptors distinguish between such subtle changes in a molecule?

We shall consider one theory which attempts to explain how these distinctions might take place, but it is important to realize that there are alternative theories. In this particular theory, it is suggested that there are two accessory hydrophobic binding sites present in an analgesic receptor. It is then proposed that a structure will act as an agonist or as an antagonist depending on which of these extra binding sites is used. In other words, one of the hydrophobic binding sites is an agonist binding site, whereas the other is an antagonist binding site.

The model was proposed by Snyder and co-workers and is shown in Figs. 12.35–12.37).[2] In the model, the agonist binding site is further away from the nitrogen and positioned axially with respect to it. The antagonist site is closer and positioned equatorially.

Let us now consider the morphine analogue containing a phenethyl substituent on the nitrogen (Fig. 12.35). It is proposed that this structure binds as already described, such that the phenol, aromatic ring, and basic centre are interacting with their respective binding sites. If the phenethyl group is in the axial position, the aromatic ring is in the correct position to interact with the agonist binding site. However, if the phenethyl group is in the equatorial position, the aromatic ring is placed beyond the antagonist binding site and cannot bind. The overall result is increased activity as an agonist.

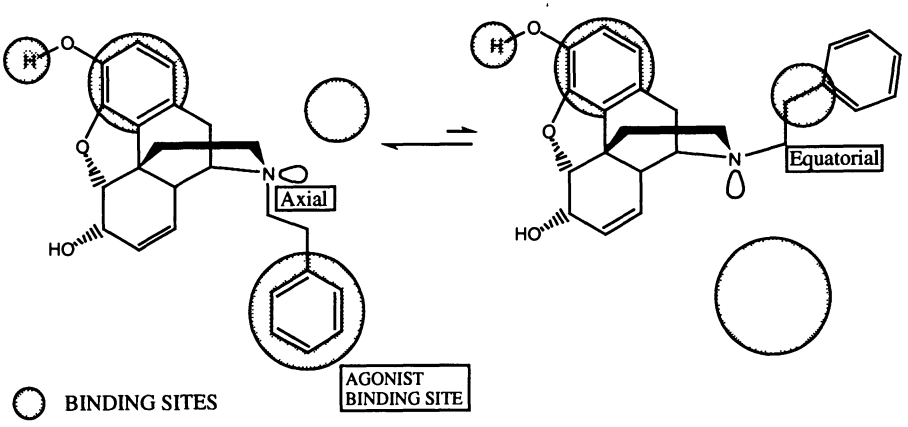


Fig. 12.35 Morphine analogue containing a phenethyl substituent on the nitrogen.

[2] Feinberg, A.P., Creese, I., and Snyder, S.H. (1976). *Proc. Natl. Acad. Sci. USA*, **73**, 4215.

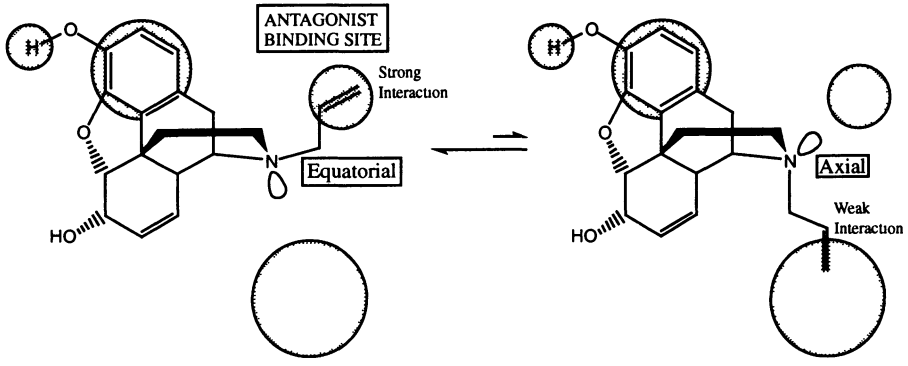


Fig. 12.36 Morphine analogue containing anallyl substituent.

Let us now consider what happens if the phenethyl group is replaced with an allyl group (Fig. 12.36). In the equatorial position, the allyl group is able to bind strongly to the antagonist binding site, whereas in the axial position it barely reaches the agonist binding site, resulting in a weak interaction.

In this theory, it is proposed that a molecule such as phenazocine (with a phenethyl group) acts as an agonist since it can only bind to the agonist binding site. A molecule such as nalorphine (with an allyl group) can bind to both agonist and antagonist sites and therefore acts as an agonist at one receptor and an antagonist at another. The ratio of these effects would depend on the relative equilibrium ratio of the axial and equatorial substituted isomers.

A compound which is a pure antagonist would be forced to have a suitable substituent in the equatorial position. It is believed that the presence of a 14-OH group sterically hinders the isomer with the axial substituent, and forces the substituent to remain equatorial (Fig. 12.37).

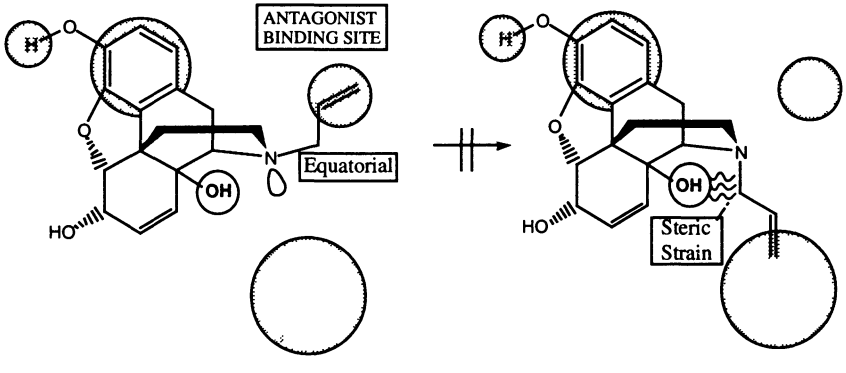


Fig. 12.37 Influence of 14-OH on binding interactions.

12.6 *Enkephalins and endorphins*

12.6.1 Naturally occurring enkaphalins and endorphins

Morphine, as we have already discussed, is an alkaloid which relieves pain and acts in the CNS. There are two conclusions which can be drawn from this. The first is that there must be analgesic receptors in the CNS. The second conclusion is that there must be chemicals produced in the body which interact with these receptors. Morphine itself is not produced by humans and therefore the body must be using a different chemical as its natural painkiller.

The search for this natural analgesic took many years, but ultimately led to the discovery of the enkephalins and the endorphins. The term enkephalin is derived from the Greek, meaning 'in the head', and that is exactly where the enkephalins are produced. The first enkephalins to be discovered were the pentapeptides Met-enkephalin and Leu-enkephalin.

H–Tyr–Gly–Gly–Phe–Met–OH H–Tyr–Gly–Gly–Phe–Leu–OH

At least 15 endogenous peptides have now been discovered, varying in length from 5 to 33 amino acids (the enkephalins and the endorphins). These compounds are thought to be neurotransmitters or neurohormones in the brain and operate as the body's natural painkillers as well as having a number of other roles. They are derived from three inactive precursor proteins—proenkephalin, prodynorphin, or pro-opiomelanocortin (Fig. 12.38).

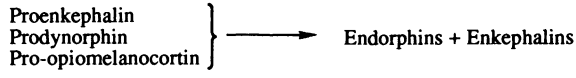


Fig. 12.38 Production of the body's natural painkillers.

All 15 compounds are found to have either the Met- or the Leu-enkephalin skeleton at their N-terminus, which emphasizes the importance of this pentapeptide structure towards analgesic activity. It has also been shown conclusively that the tyrosine part of these molecules is essential to activity and much has been made of the fact that there is a tyrosine skeleton in the morphine skeleton (Fig. 12.39).

Enkephalins are thought to be responsible for the analgesia resulting from acupuncture.

12.6.2 Analogues of enkephalins

SAR studies on the enkephalins have shown the importance of the tyrosine phenol ring and the tyrosine amino group. Without either, activity is lost. If tyrosine is replaced with another amino acid, then activity is also lost (the only exception being D-serine). It has also been found that the enkephalins are easily inactivated by

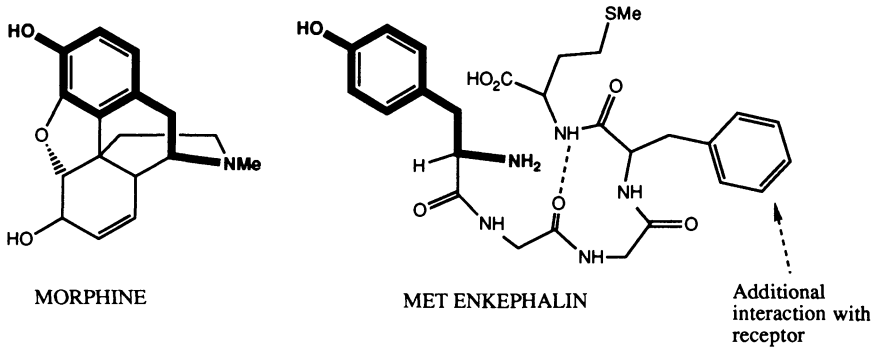


Fig. 12.39 The tyrosine section is essential to activity.

peptidase enzymes *in vivo*. The most labile peptide bond in the enkephalins is that between the tyrosine and glycine residues.

Much work has been done therefore, to try and stabilize this bond towards hydrolysis. It is possible to replace the amino acid glycine with an unnatural D-amino acid such as D-alanine. Since D-amino acids are not naturally occurring, peptidases do not recognize the structure and the peptide bond is not attacked. The alternative tactic of replacing L-tyrosine with D-tyrosine is not possible, since this completely alters the relative orientation of the tyrosine aromatic ring with respect to the rest of the molecule. As a result, the analogue is unable to bind to the analgesic receptor and is inactive.

Putting a methyl group on to the amide nitrogen can also block hydrolysis by peptidases. Another tactic is to use unusual amino acids which are either not recognized by peptidases or prevent the molecule from fitting the peptidase active site. Examples of these tactics at work are demonstrated in Fig. 12.40.

Unfortunately, the enkephalins also have some activity at the mu receptor and so the search for selective agents continues.

H—L-Tyr—Gly—Gly—L-Phe—L-Met—OH	Delta Agonist + a little mu activity
H—L-Tyr—D-AA—Gly—NMe-L-Phe—L-Met—OH	Resistant to peptidase. Orally active.
N,N-Diallyl-L-Tyr—aib—aib—L-Phe—L-Leu—OH	Antagonist to delta receptor. (aib = alpha-aminobutyric acid)
Longer enkaphalins/endorphins	Increase in kappa activity Slight increase in mu activity

Fig. 12.40 Tactics to stabilize the bond between the tyrosine and glycine residues.

12.7 *Receptor mechanisms*

Up until now we have discussed receptors very much as 'black boxes'. The substrate comes along, binds to the receptor, and switches it on. There is a biological response,

be it analgesia, sedation, or whatever, but we have given no indication of how this response takes place. Why should morphine cause analgesia just by attaching itself to a receptor protein?

In general, all receptors in the body are situated on the surface of cells and act as communication centres for the various messages being sent from one part of the body to another. The message may be sent through nerves or via hormones, but ultimately the message has to be delivered from one cell to another by a chemical messenger. This chemical messenger has to 'dock' with the receptor which is waiting for it. When it does so, it forces the receptor to change shape. This change in shape of the receptor molecule may force a change in the shape of some neighbouring protein or perhaps an ion channel, resulting in an alteration of ion flows in and out of the cell. Such effects will ultimately have a biological effect, dependent on the cells affected.

We shall now look at the analgesic receptors in a little more detail.

12.7.1 The mu receptor (μ)

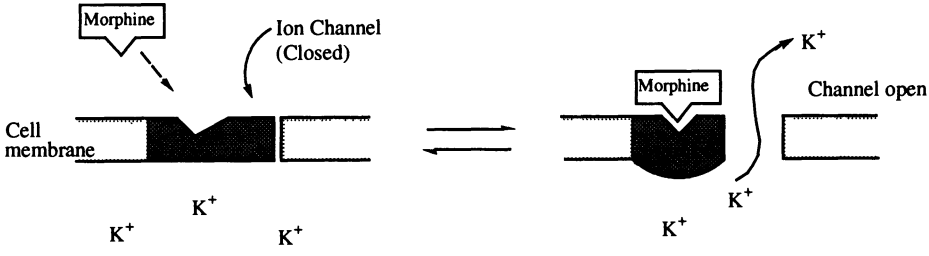


Fig. 12.41 Morphine binding to μ receptor.

As the diagram in Fig. 12.41 demonstrates, morphine binds to the μ receptor and induces a change in shape. This change in conformation opens up an ion channel in the cell membrane and as a result, potassium ions can flow out of the cell. This flow hyperpolarizes the membrane potential and makes it more difficult for an active potential to be reached (see Appendix 2).

Therefore, the frequency of action potential firing is decreased, which results in a decrease in neurone excitability.

This increase in potassium permeability has an indirect effect, since it also decreases the influx of calcium ions into the nerve terminal and this in turn reduces neurotransmitter release.

Both effects, therefore, 'shut down' the nerve and block the pain messages.

Unfortunately, this receptor is also associated with the hazardous side-effects of

narcotic analgesics. There is still a search to see if there are possibly two slightly different mu receptors, one which is solely due to analgesia and one responsible for the side-effects.

12.7.2 The kappa receptor (κ)

The κ receptor is directly associated with a calcium channel (Fig. 12.42). When an agonist binds to the κ receptor, the receptor changes conformation and the calcium channel (normally open when the nerve is firing and passing on pain messages) is closed. Calcium is required for the production of the nerves neurotransmitters and therefore the nerve is shut down and cannot pass on pain messages.

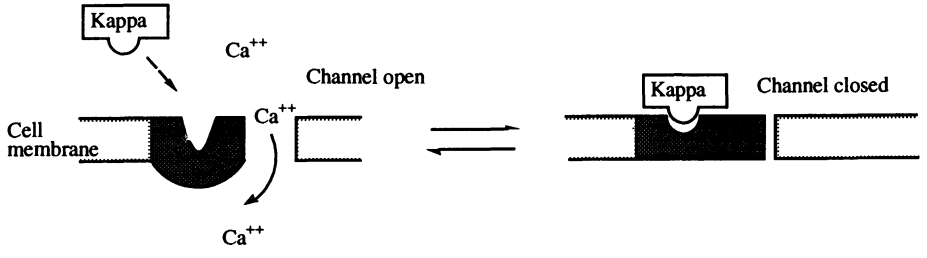


Fig. 12.42 The association of the κ receptor and calcium channels.

The nerves affected by the κ mechanism are those related to pain induced by non-thermal stimuli. This is not the case with the μ receptor where all pain messages are inhibited. This suggests a different distribution of κ receptors from μ receptors.

12.7.3 The delta receptor (Δ)

Like the μ receptor, the nerves containing the Δ receptor do not discriminate between pain from different sources.

In this case, there are no ion channels involved (Fig. 12.43). The substrate molecule binds to the Δ receptor and, in some way, the message is transmitted through the cell

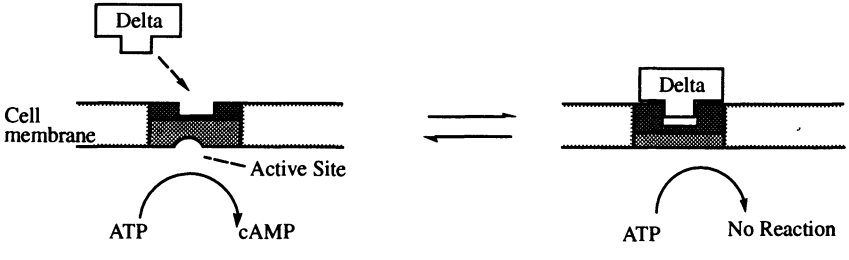


Fig. 12.43 The delta receptor.

membrane to a second membrane-bound protein. This protein then acts as an enzyme for the formation of cyclic AMP (Fig. 12.44). Normally, the active site is open when the nerve is receiving pain messages, such that cyclic AMP acts as a secondary messenger and passes on the pain messages. However, when the Δ receptor is activated it probably changes shape and as a result leads to a change in the shape of the cyclase enzyme to close down the active site by which it can make cyclic AMP (see also Appendix 3).

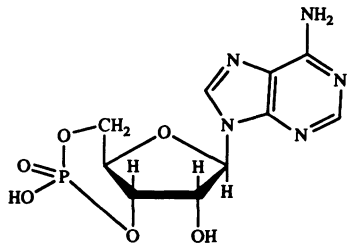


Fig. 12.44 Cyclic adenosine monophosphate (cAMP).

12.7.4 The sigma receptor (σ)

This receptor is not an analgesic receptor, but we have seen that it can be activated by certain opiate molecules such as nalorphine. When activated, it produces hallucinogenic effects. The σ receptor may be the one associated with the hallucinogenic and psychotomimetic effects of phencyclidine (PCP), otherwise known as 'angel dust' (Fig. 12.45).

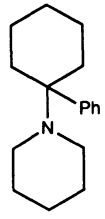
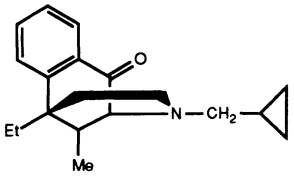


Fig. 12.45 Phencyclidine (PCP) or 'angel dust'.

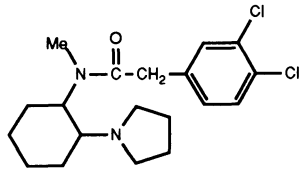
12.8 *The future*

There is still a need for analgesic drugs with reduced side-effects. Four approaches are feasible in the field of opiates.

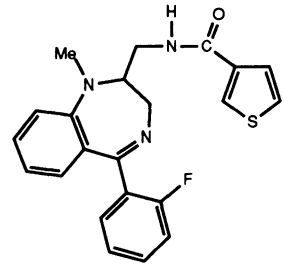
- κ Agonists.
  Such compounds should have much-reduced side-effects. However, a completely specific κ agonist has not yet been found and there may be a close link between the Δ receptor and the σ receptor. Examples of selective agonists are shown in Fig. 12.46.
- Selectivity between mu receptor subtypes.
  There might be two slightly different μ receptors, one of which is purely responsible for analgesia (μ_1) and the other solely responsible for unwanted side-effects such as respiratory depression (μ_2). An agent showing selectivity would prove such a theory and be very useful analgesic.



Ethylketocyclazocine
(Kappa=mu > delta)



U 50488
(kappa > mu)



Tifluadom
(kappa > mu)

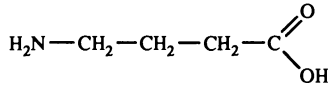
Fig. 12.46 Examples of selective agonists.

- Peripheral opiate receptors.
  Peripheral opiate receptors have been identified in the ileum and are responsible for the antidiarrhoeal activity of opiates. If peripheral sensory nerves also possess opiate receptors, drugs might be designed versus these sites and as a result would not need to cross the blood–brain barrier.
- Blocking postsynaptic receptors.
  Perhaps blocking the chemical messengers from transmitting pain information by blocking postsynaptic receptors with selective antagonists would be the best approach. This would involve non-opioids and non-opioid mechanisms, but might be the best way of eliminating side-effects.

One promising lead is provided by the neurotransmitter GABA (Fig. 12.47) which appears to have a role in the regulation of enkephalinergic neurons and as such affects pain pathways.

An undecapeptide called substance P is an excitatory neurotransmitter which appears to have a role in pain mediation and is worthy of further study.

Fig. 12.47 γ-aminobutanoic acid (GABA).



13 ▪ Cimetidine—a rational approach to drug design

13.1 *Introduction*

Many of the past successes in medicinal chemistry have involved the fortuitous discovery of useful pharmaceutical agents from natural sources such as plants or microorganisms. Analogues of these structures were then made in an effort to improve activity and/or to reduce side-effects, but often these variations were carried out on a trial-and-error basis. While this approach yielded a large range of medicinal compounds, it was wasteful with respect to the time and effort involved.

In the last twenty to thirty years, greater emphasis has been placed on rational drug design whereby drugs are designed to interact with a known biological system. For example, when looking for an enzyme inhibitor, a rational approach is to purify the enzyme and to study its tertiary structure by X-ray crystallography. If the enzyme can be crystallized along with a bound inhibitor, then the researcher can identify and study the binding site of the enzyme. The X-ray data can be read into a computer and the binding site studied to see whether new inhibitors can be designed to fit more strongly.

However, it is not often possible to isolate and purify enzymes, and when it comes to membrane-bound receptors, the difficulties become even greater. Nevertheless, rational drug design is still possible, even when the receptor cannot be studied directly. The strengths of the rational approach to drug design are amply demonstrated by the development of the antiulcer drug cimetidine (Tagamet) (Fig. 13.1), carried out by scientists at Smith, Kline, & French, (SK&F).

The remarkable aspect of the cimetidine story lies in the fact that at the onset of the

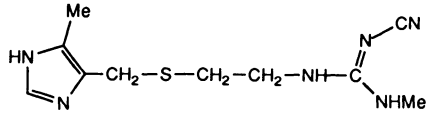


Fig. 13.1 Cimetidine.

project there were no lead compounds and it was not even known if the necessary receptor protein even existed!

13.2 *In the beginning—ulcer therapy in 1964*

When the cimetidine programme started in 1964, the methods available for treating peptic ulcers were few and generally unsatisfactory.

Ulcers are localized erosions of the mucous membranes of the stomach or duodenum. It is not known how these ulcers arise, but the presence of gastric acid aggravates the problem and delays recovery. In the early 1960s, the conventional treatment was to try and neutralize gastric acid in the stomach by administering bases such as sodium bicarbonate or calcium carbonate. However, the dose levels required for neutralization were large and caused unpleasant side-effects. It was reasoned that a better approach would be to inhibit the release of gastric acid at source.

Gastric acid (HCl) is released by cells known as parietal cells in the stomach (Fig. 13.2). These parietal cells are innervated with nerves (not shown on the diagram) from the automatic nervous system (see Chapter 11). When the autonomic nervous system is stimulated, a signal is sent to the parietal cells culminating in the release of the neurotransmitter acetylcholine at the nerve termini. Acetylcholine crosses the gap between nerve and parietal cell and activates the cholinergic receptors of the parietal cells leading to the release of gastric acid into the stomach. The trigger for this process is provided by the sight, smell, or even the thought, of food. Thus, gastric acid is released before food has even entered the stomach.

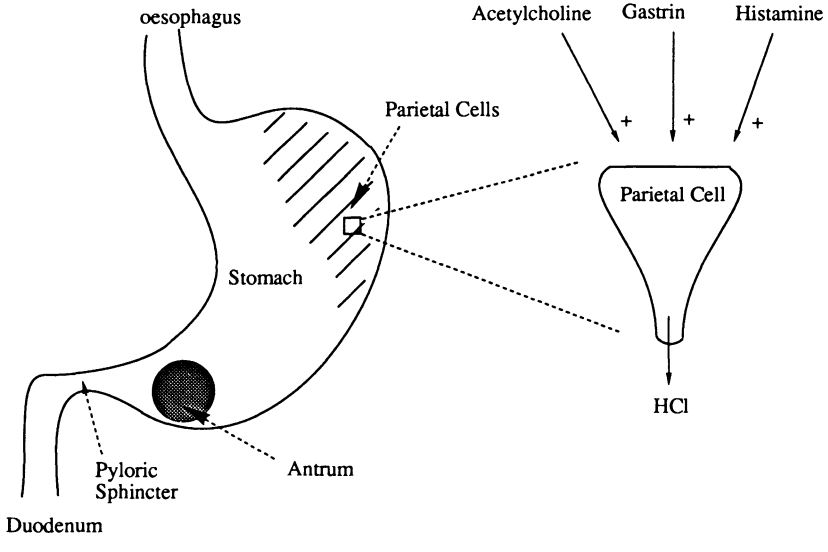


Fig. 13.2 The stomach.

Nerve signals also stimulate a region of the stomach known as the antrum which contains hormone-producing cells known as G cells. The hormone released is a peptide called gastrin (Fig. 13.3) which is also released when food is present in the stomach. The gastrin moves into the blood supply and travels to the parietal cells further stimulating the release of gastric acid. Release of gastric acid should therefore be inhibited by antagonists blocking either the acetylcholine receptor or the receptor for gastrin.

Fig. 13.3 Gastrin. p-Glu-Gly-Pro-Trp-Leu-$[Glu]_5$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

Agents which block the acetylcholine receptor are known as anticholinergic drugs (see Chapter 11). These agents certainly block the cholinergic receptor in parietal cells and inhibit release of gastric acid. Unfortunately, they also inhibit acetylcholine receptors at other parts of the body and cause unwanted side-effects.

Therefore, in 1964, the best hope of achieving an antiulcer agent appeared to be in finding a drug which would block the hormone gastrin. Several research teams were active in this field, but the research team at SK&F decided to follow a different tack altogether.

It was known that histamine (Fig. 13.4) could also stimulate gastric acid release, and it was proposed by the SK&F team that an antihistamine agent might also be effective in treating ulcers. At the time, this was a highly speculative proposal. Although histamine had been shown experimentally to stimulate gastric acid release, it was by no means certain that it played any significant role *in vivo*. Many workers at the time discounted the importance of histamine, especially when it was found that conventional antihistamines failed to inhibit gastric acid release. This result appeared to suggest the absence of histamine receptors in the parietal cells. The fact that histamine did have a stimulatory effect could be explained away by suggesting that histamine coincidentally switched on the gastrin or acetylcholine receptors.

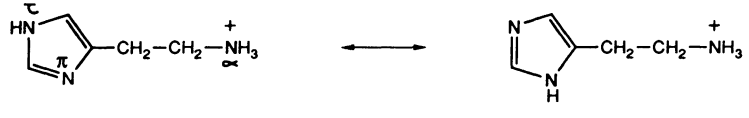


Fig. 13.4 Histamine.

Why then did the SK&F team persevere in their search for an effective antihistamine? What was their reasoning? Before answering that, let us look at histamine itself and the antihistamines available at that time.

13.3 *Histamine*

Histamine is made up of an imidazole ring which can exist in two tautomeric forms as shown in Fig. 13.4. Attached to the imidazole ring there is a two-carbon chain with a terminal α-amino group. The pK_a of this amino group is 9.80, which means that at a plasma pH of 7.4, the side-chain of histamine is 99.6 per cent ionized.

The pK_a of the imidazole ring is 5.74 and so the ring is mostly un-ionized at pH 7.4.

Whenever cell damage occurs, histamine is released and stimulates the dilation and increased permeability of small blood vessels. The advantage of this to the body is that defensive cells (e.g. white blood cells) are released from the blood supply into an area of tissue damage and are able to combat any potential infection. Unfortunately, the release of histamine can also be a problem. For example, when an allergic reaction or irritation is experienced, histamine is released and produces the same effects when they are not really needed.

The early antihistamine drugs were therefore designed to treat conditions such as hay fever, rashes, insect bites, or asthma.

Two examples of these early antihistamines are mepyramine (Fig. 13.5) and diphenhydramine ('Benadryl') (Fig. 13.6).

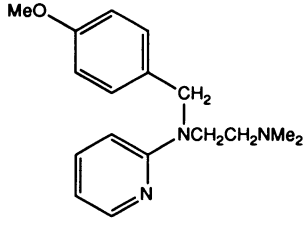


Fig. 13.5 Mepyramine.

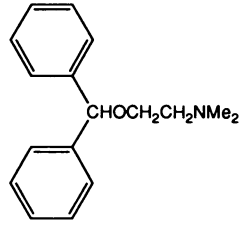


Fig. 13.6 Diphenhydramine.

13.4 *The theory—two histamine receptors?*

We are now able to return to the question we asked at the end of Section 13.2. Bearing in mind the failure of the known antihistamines to inhibit gastric acid release, why did the SK&F team persevere with the antihistamine approach?

As mentioned above, conventional antihistamines failed to have any effect on gastric acid release. However, they also failed to inhibit other actions of histamine. For example, they failed to fully inhibit the dilation of blood vessels induced by histamine. The SK&F scientists therefore proposed that there might be two different types of histamine receptor, analogous to the two types of acetylcholine receptor mentioned in Chapter 11. Histamine—the natural messenger—would switch both on equally effectively and would not distinguish between them. However, suitably

designed antagonists should in theory be capable of making that distinction. By implication, this meant that the conventional antihistamines known in the early sixties were already selective in that they were able to inhibit the histamine receptors involved in the inflammation process (classified as H1 receptors), and were unable to inhibit the proposed histamine receptors responsible for gastric acid secretion (classified as H2 receptors).

It was an interesting theory, but the fact remained that there was no known antagonist for the proposed H2 receptors. Until such a compound was found, it could not be certain that the H2 receptors even existed, and yet without a receptor to study, how could one design an antagonist to act with it?

13.5 *Searching for a lead—histamine*

The SK&F team obviously had a problem. They had a theory but no lead compound. How could they make a start?

Their answer was to start from histamine itself. If histamine was stimulating the release of gastric acid by binding to a hypothetical H2 receptor, then clearly histamine was being 'recognized' by the receptor. The task then was to vary the structure of histamine in such a way that it would still be recognized by the receptor, but bind in such a way that it acted as an antagonist rather than an agonist.

It was necessary then to find out how histamine itself was binding to its receptors. Structure activity studies on histamine and histamine analogues revealed that the binding requirements for histamine to the H1 and the proposed H2 receptors were slightly different.

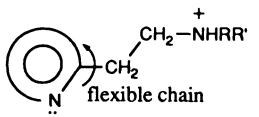
At the H1 receptor, the essential requirements were as follows:

- The side-chain had to have a positively charged nitrogen atom with at least one attached proton. Quaternary ammonium salts which lacked such a proton were extremely weak in activity.
- There had to be a flexible chain between the above cation and a heteroaromatic ring.
- The heteroaromatic ring did not have to be imidazole, but it did have to contain a nitrogen atom with a lone pair of electrons, *ortho* to the side-chain.

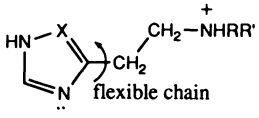
For the proposed H2 receptor, structure–activity studies were carried out to determine whether histamine analogues could bring about the physiological effects proposed for this receptor (e.g. stimulating gastric acid release).

The essential structure–activity requirements were the same as for the H1 receptor except that the heteroaromatic ring had to contain an amidine unit (HN–CH–N:). These results are summarized in Fig. 13.7.

From these results, it appeared that the terminal α-amino group was involved in a binding interaction with both types of receptor via ionic or hydrogen bonding, while



SAR for Agonist at H1 Receptor



SAR for Agonist at proposed H2 Receptor

Fig. 13.7 Summary of SAR results.

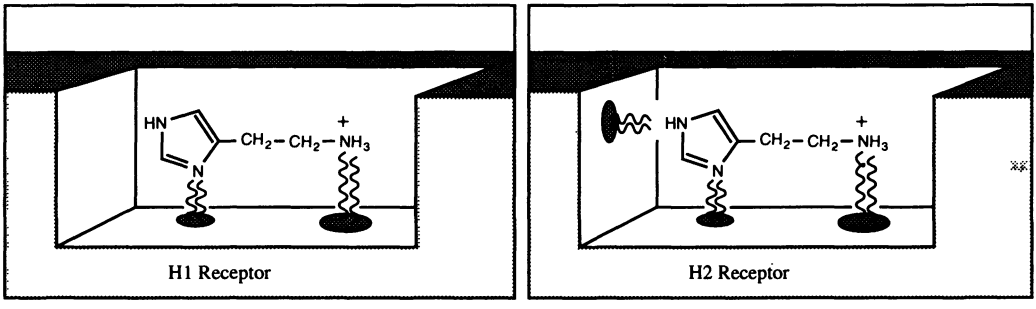


Fig. 13.8 Binding interactions for H1 and H2.

the nitrogen atom(s) in the heteroaromatic ring bound via hydrogen bonding, as shown in Fig. 13.8.

13.6 *Searching for a lead—N$^{\alpha}$-guanylhistamine*

Having gained a knowledge of the structure–activity relationships for histamine, the task was now to design a molecule which would be recognized by the H2 receptor, but which would not activate it. In other words, an agonist had to be converted to an antagonist. In order to do that, it would be necessary to alter the way in which the molecule was bound to the receptor.

Pictorially, one can imagine histamine fitting into its receptor site and inducing a change in shape which 'switches the receptor on' (Fig. 13.9). An antagonist might be found by adding a functional group which would bind to another binding site on the receptor and prevent the change in shape required for activation.

This was one of several strategies tried out by the SK&F workers. To begin with, a study of known agonists and antagonists in other fields of medicinal chemistry was carried out. The structural differences between agonists and antagonists for a particular receptor were identified and then similar alterations were tried on histamine.

For example, fusing an aromatic ring on to noradrenaline had been a successful tactic used in the design of antagonists for the noradrenaline receptor (see Section 7.5.5.). This same tactic was tried with histamine to give analogues such as the one shown in Fig. 13.10, but none of the compounds synthesized proved to be an antagonist.

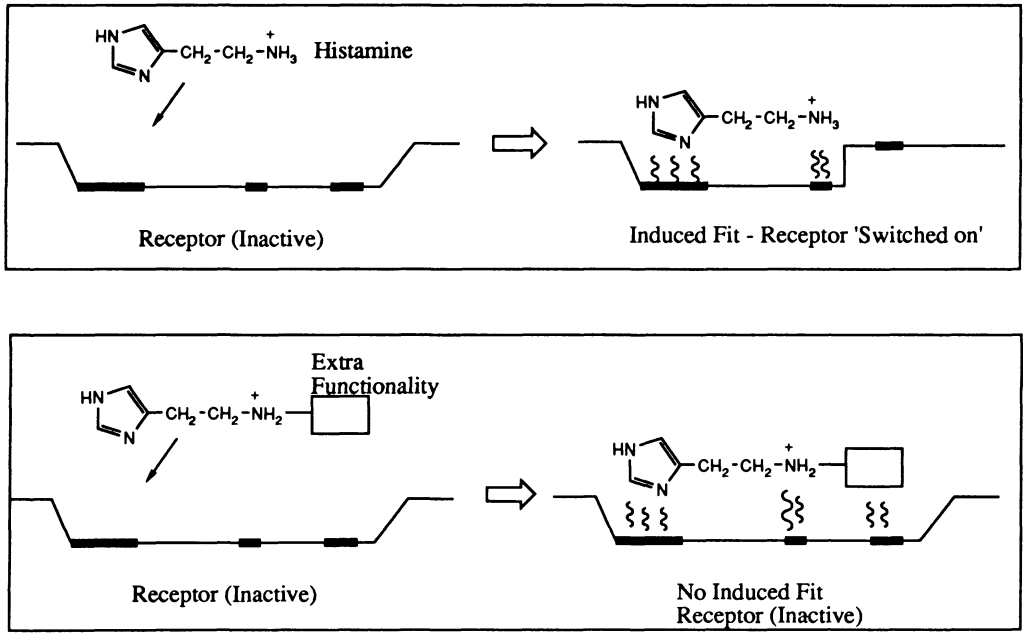


Fig. 13.9 Possible receptor interactions of histamine and an antagonist.

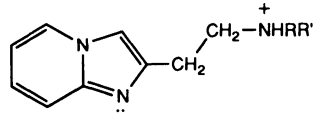


Fig. 13.10 Histamine analogue—not an antagonist.

Another approach which had been used successfully in the development of anti-cholinergic agents (section 11.11.2.) had been the addition of non-polar, hydrophobic substituents. This approach was tried with histamine by attaching various alkyl and arylalkyl groups to different locations on the histamine skeleton. Unfortunately, none of these analogues proved to be antagonists.

However, one interesting result was obtained which was to be relevant to later studies. It was discovered that 4-methylhistamine (Fig 13.11) was a highly selective

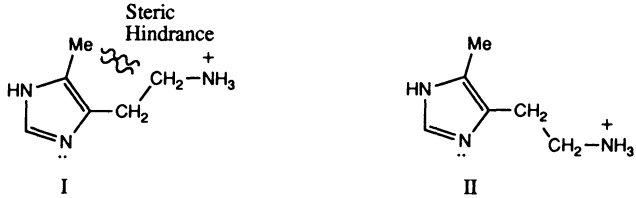


Fig. 13.11 4-Methylhistamine.

H2 agonist, showing far greater activity for the H2 receptor than for the H1 receptor. Why should such a simple alteration produce this selectivity?

4-Methylhistamine (like histamine) is a highly flexible molecule due to its side-chain, but structural studies show that some of its conformations are less stable than others. In particular, conformation I in Fig. 13.11 is disallowed due to a large steric interaction between the 4-methyl group and the side-chain. The selectivity observed suggests that 4-methylhistamine (and by inference histamine) has to adopt two different conformations in order to fit the H1 or the H2 receptor. Since 4-methylhistamine is more active at the H2 receptor, it implies that the conformation required for the H2 receptor is a stable one for 4-methylhistamine (conformation II), whereas the conformation required for the H1 receptor is an unstable one (conformation I).

Despite this interesting result, the SK&F workers were no closer to an H2 antagonist. Two hundred compounds had been synthesized and not one had shown a hint of being an antagonist.

The work up until this stage had concentrated on searching for an additional hydrophobic binding site on the receptor. Now the focus of research switched to see what would happen if the terminal αNH_3^+ group was replaced with a variety of different polar functional groups. It was reasoned that different polar groups could bond to the same site on the receptor as the NH_3^+ group, but that the geometry of bonding might be altered sufficiently to produce an antagonist. It was from this study that the first crucial breakthrough was achieved with the discovery that N^{α}-guanylhistamine (Fig. 13.12) was acting very weakly as an antagonist.

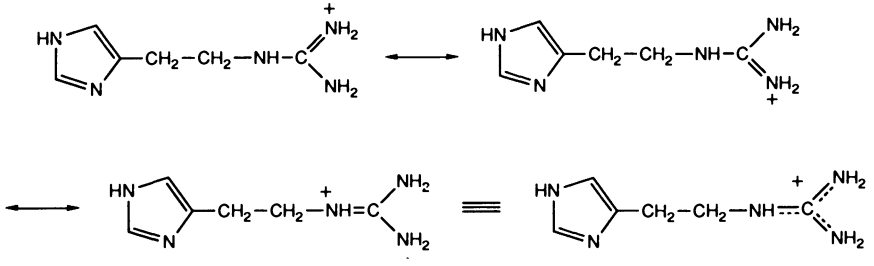


Fig. 13.12 N^{α}-Guanylhistamine.

This structure had in fact been synthesized early on in the project, but had not been recognized as an antagonist. This is not too surprising since it acts as an agonist! It was not until later pharmacological studies were carried out that it was realized that N^{α}-guanylhistamine was also acting as an antagonist to histamine. In other words, it was a partial agonist (see Section 5.8.).

N^{α}-guanylhistamine activates the H2 receptor, but not to the same extent as histamine. As a result, the amount of gastric acid released is lower. More importantly,

as long as N^{α}-guanylhistamine is bound to the receptor, it prevents histamine from binding and thus prevents complete receptor activation.

This was the first indication of any sort of antagonism to histamine.

The question now arose as to which part or parts of the N^{α}-guanylhistamine skeleton were really necessary for this effect. Perhaps the guanidine group itself could act as an antagonist?

Various guanidine structures were synthesized which lacked the imidazole ring, but none had the desired antagonist activity, demonstrating that both the imidazole ring and the guanidine group were required.

The structures of N^{α}-guanylhistamine and histamine were now compared. Both structures contain an imidazole ring and a positively charged group linked by a two-carbon bridge. The guanidine group is basic and protonated at pH 7.4 so that the analogue has a positive charge similar to histamine. However, the charge on the guanidine group can be spread around a planar arrangement of three nitrogens and can potentially be further away from the imidazole ring (Fig. 13.12). This leads to the possibility that the analogue could be interacting with another binding group on the receptor which is 'out of reach' of histamine. This is demonstrated in Fig. 13.13 and 13.14. Two alternative binding sites might be available for the cationic group—an agonist site where binding leads to activation of the receptor and an antagonist site where binding does not activate the receptor. In Fig. 13.14, histamine is only able to

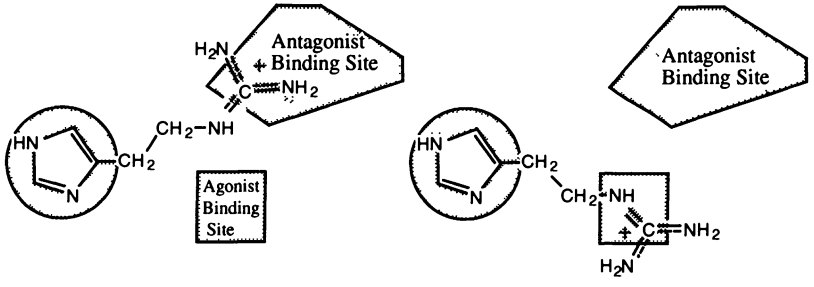


Fig. 13.13 Possible binding modes for N^{α}-guanylhistamine.

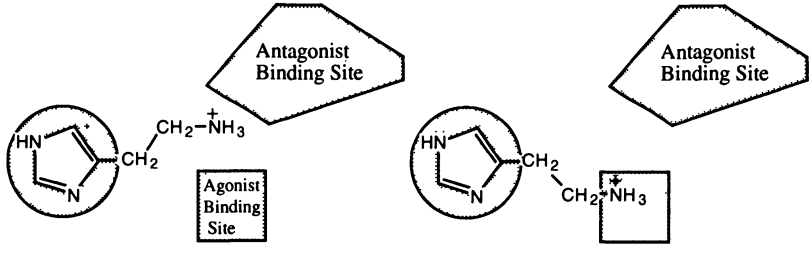


Fig. 13.14 Binding of histamine: agonist mode only.

reach the agonist site. However, the analogue with its extended functionality is capable of reaching either site (Fig. 13.13).

If most of the analogue molecules bind to the agonist site and the remainder bind to the antagonist site, then this could explain the partial agonist activity. Regardless of the mode of binding, histamine would be prevented from binding and an antagonism would be observed due to the percentage of N^{α}-guanylhistamine bound to the antagonist site.

13.7 *Developing the lead—a chelation bonding theory*

Variations were now necessary to see if an analogue could be made which would only bind to the antagonist site.

The synthesis of the isothiourea (Fig. 13.15) gave a structure where the nitrogen nearest to the imidazole ring was replaced with a sulfur atom.

The positive charge in this molecule is now restricted to the terminal portion of the chain and should interact more strongly with the proposed antagonist binding site if it is indeed further away.

Antagonist activity did increase, but the compound was still a partial agonist, showing that binding was still possible to the agonist site.

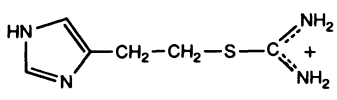


Fig. 13.15 Isothiourea analogue.

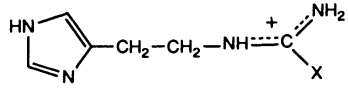


Fig. 13.16 Analogue, where X is a methylthio group or methyl group.

Two other analogues were synthesized, where one of the terminal amino groups in the guanidine group was replaced with either a methylthio group or a methyl group (Fig. 13.16). Both the resulting structures was partial agonists, but with poorer antagonist activity.

From these results, it was concluded that both terminal amino groups were required for binding to the antagonist binding site. It was proposed that the charged guanidine group was interacting with a charged carboxylate residue on the receptor via two hydrogen bonds (Fig. 13.17). If either of these terminal amino groups were absent, then binding would be weaker, resulting in a lower level of antagonism.

The chain was now extended from a two-carbon unit to a three-carbon unit to see what would happen if the guanidine group was moved further away from the imidazole ring. The antagonist activity increased for the guanidine structure (Fig. 13.18), but

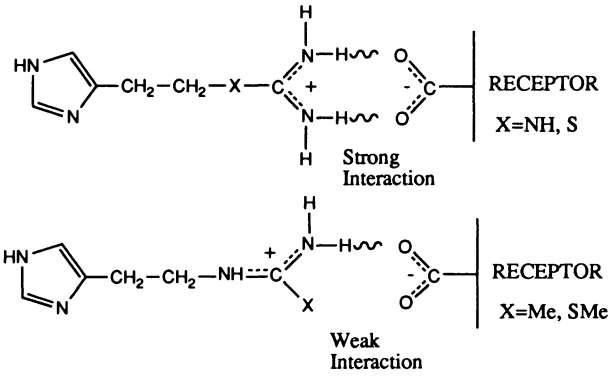


Fig. 13.17 Proposed interaction of the charged guanidine group.

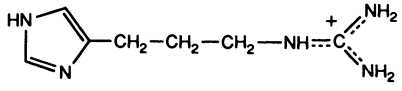


Fig. 13.18 Guanidine structure.

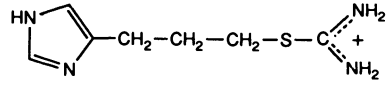


Fig. 13.19 Isothiourea structure.

strangely enough, decreased for the isothiourea structure (Fig. 13.19). It was therefore proposed that with a chain length of two carbon units, hydrogen bonding to the receptor involved the terminal NH_2 groups, but with a chain length of three carbon units, hydrogen bonding involved one terminal NH_2 group along with the NH group within the chain (Fig. 13.20). Support for this theory was provided by the fact that replacing one of the terminal NH_2 groups in the guanidine analogue (Fig. 13.18) with SMe or Me (Fig. 13.21) did not adversely affect the antagonist activity. This was completely different from the results obtained when similar changes were carried out on the two-carbon bridged compound.

These bonding interactions are represented pictorially in Figs. 13.22 and 13.23.

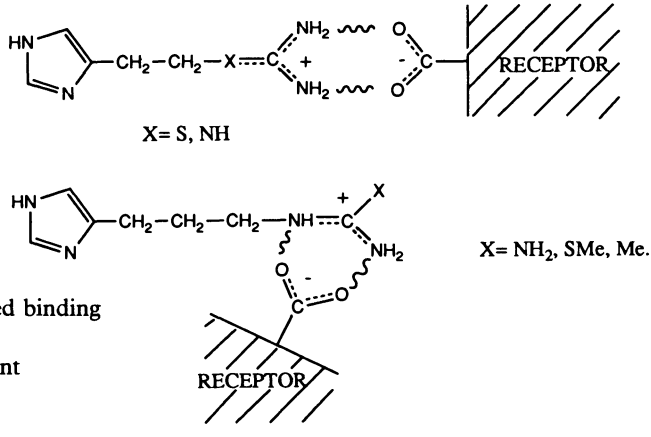


Fig. 13.20 Proposed binding interactions for analogues of different chain length.

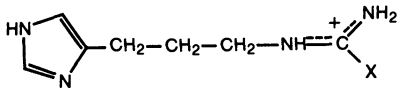


Fig. 13.21 Guanidine analogue with SMe or Me.

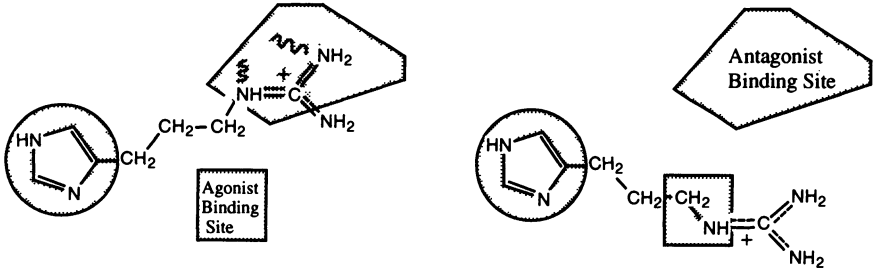


Fig. 13.22 Binding interactions for 3C bridged analogue.

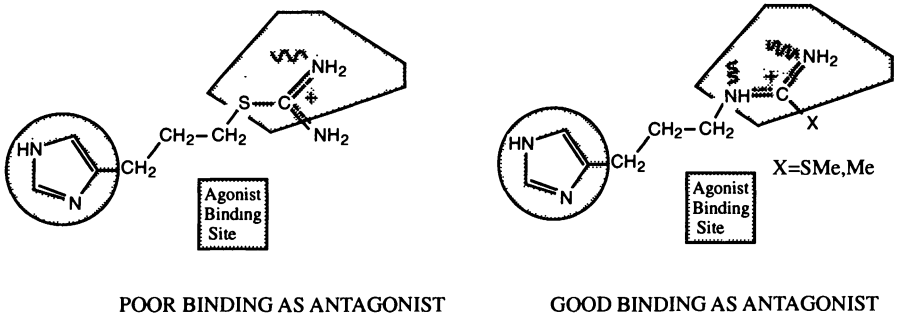


Fig. 13.23 Effect of varying the guanidine group on binding to the antagonist site.

13.8 *From partial agonist to antagonist—the development of burimamide*

The problem now was to completely remove the agonist activity to get compounds with pure antagonist activity. This meant designing a structure which would differentiate between the agonist and antagonist binding sites.

At first sight this looks impossible since both sites appear to involve the same type of bonding. Histamine's activity as an agonist depends on the imidazole ring and the charged amino function, with the two groups taking part in hydrogen and ionic bonding, respectively. However, the antagonist activity of the partial agonists described so far also appear to depend on a hydrogen bonding imidazole ring and an ionic bonding guanidine group.

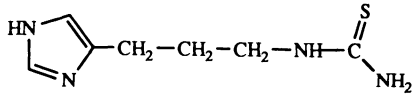
Fortunately, a distinction can be made between the charged groups.

The structures which show antagonist activity are all capable of forming a chelated bonding structure as previously shown in Fig. 13.20. This interaction involves two hydrogen bonds between two charged species, but is it really necessary for the chelating group to be charged? Could a neutral group also chelate to the antagonist site by hydrogen bonding alone? If so, it might be possible to distinguish between the agonist and antagonist sites, especially since ionic bonding appears necessary for the agonist site.

It was therefore decided to see what would happen if the strongly basic guanidine group was replaced with a neutral group capable of interacting with the receptor by two hydrogen bonds. There are a large variety of such groups, but the SK&F workers limited the options by adhering to a principle which they followed throughout their research programme. Whenever they wished to alter any specific physical or chemical property, they strove to ensure that other properties were changed as little as possible. Only in this way could they rationalize any observed improvement in activity.

Thus, in order to study the effect of replacing the basic guanidine group with a neutral group, it was necessary to ensure that the new group was as similar as possible to guanidine in terms of size, shape, and hydrophobicity.

Several functional groups were tried, but success was ultimately achieved by using a thiourea group. The thiourea derivative SK&F 91581 (Fig. 13.24) proved to be a weak antagonist with no agonist activity.



No Agonist Activity
Very Weak Antagonist Activity

Fig. 13.24 SK&F 91581.

Apart from basicity, the properties of the thiourea group are very similar to the guanidine group. Both groups are planar, similar in size, and can take part in hydrogen bonding. Thus, the alteration in biological activity can reasonably be attributed to the differences in basicity between the two groups.

Unlike guanidine, the thiourea group is neutral. This is due to the C=S group which has an electron withdrawing effect on the neighbouring nitrogens, making them non-basic and more like amide nitrogens.

The fact that a neutral group could bind to the antagonist site and not to the agonist site could be taken to imply that the agonist binding site involves ionic bonding, whereas the antagonist site involves hydrogen bonding.

Further chain extension and the addition of an *N*-methyl group led to burimamide (Fig. 13.25) which was found to have enhanced activity.

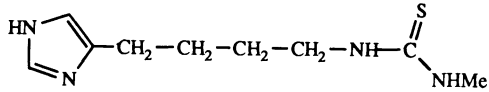


Fig. 13.25 Burimamide.

These results suggest that chain extension has moved the thiourea group closer to the antagonist binding site, and that the addition of the *N*-methyl group has resulted in a beneficial increase in hydrophobicity. A possible explanation for this latter result will be described in Section 13.12.2.

Burimamide is a highly specific competitive antagonist of histamine at H2 receptors, and is 100 times more potent than N^{α}-guanylhistamine. Its discovery finally proved the existence of the H2 receptors.

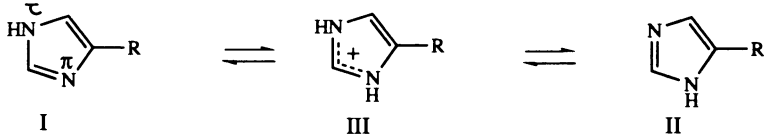
13.9 *Development of metiamide*

Despite this success, burimamide was not suitable for clinical trials since its antagonist activity was still too low for oral administration. Further developments were needed. Attention was now directed to the imidazole ring of burimamide and, in particular, to the various possible tautomeric forms of this ring. It was argued that if one particular tautomer was preferred for binding with the H2 receptor, then activity might be enhanced by modifying the burimamide structure to favour that tautomer.

At pH 7.4, it is possible for the imidazole ring to equilibrate between the two tautomeric forms (I) and (II) via the protonated intermediate (III) (Fig. 13.26). The necessary proton for this process has to be supplied by water or by an exchangeable proton on a suitable amino acid residue in the binding site. If the exchange is slow, then it is possible that the drug will enter and leave the receptor at a faster rate than the equilibration between the three tautomeric forms. If bonding involves only one of the tautomeric forms, then clearly antagonism would be increased if the structure was varied to prefer that tautomeric form over the others. Our model hypothesis for receptor binding shows that the imidazole ring is important for the binding of both agonists and antagonists. Therefore, it is reasonable to assume that the preferred imidazole tautomer is the same for both agonists and antagonists. If this is so, then the preferred tautomer for a strong agonist such as histamine should also be the preferred tautomer for a strong antagonist.

Figure 13.26 shows that the imidazole ring can exist as one ionized tautomer and two unionized tautomers. Let us first consider whether the preferred tautomer is likely to be ionized or not.

We have already seen that the pK_a for the imidazole ring in histamine is 5.74,



I III II

Fig. 13.26 Imidazole ring can equilibriate between tautomeric forms (I and II) via the protonated intermediate (III).

meaning that the ring is a weak base and mostly un-ionized. The pK_a value for imidazole itself is 6.80 and for burimamide 7.25. These values show that these imidazole rings are more basic than histamine and more likely to be ionized. Why should this be so?

The explanation must be that the side-chain has an electronic effect on the imidazole ring. If the side-chain is electron withdrawing or electron donating, then it will affect the basicity of the ring. A measure of the side-chain's electronic effect can be worked out by the Hammett equation (see Chapter 9):

$$pK_{a(R)} = pK_{a(H)} + \rho\sigma_R$$

where $pK_{a(R)}$ is the pK_a of the imidazole ring bearing a side-chain R, $pK_{a(H)}$ is the pK_a of the unsubstituted imidazole ring, ρ is a constant, and σ_R is the Hammett substituent constant for the side-chain R.

From the pK_a values, the value of the Hammett substituent constant can be calculated to show whether the side-chain R is electron withdrawing or electron donating.

In burimamide, the side-chain is calculated to be slightly electron donating (of the same order as a methyl group). Therefore, the imidazole ring in burimamide is more likely to be ionized than in histamine, where the side-chain is electron withdrawing. At pH 7.4, 40 per cent of burimamide is ionized in the imidazole ring compared to approximately 3 per cent of histamine. This represents quite a difference between the two structures and since the binding of the imidazole ring is important for antagonist activity as well as agonist activity, it suggests that a pK_a value closer to that of histamine might lead to better binding and to better antagonist activity.

It was necessary, therefore, to make the side-chain electron withdrawing rather than electron donating. This can be done by inserting an electronegative aom into the side-chain—preferably one which has a minimum disturbance on the rest of the molecule. In other words, an isostere for a methylene group is required—one which has an electronic effect, but which has approximately the same size and properties as the methylene group.

The first isostere to be tried was a sulfur atom. Sulfur is quite a good isostere for the methylene unit in that both groups have similar van der Waals radii and similar bond angles. However, the C–S bond length is slightly longer than a C–C bond, leading to a slight extension (15 per cent) of the structure.

The methylene group replaced was next but one to the imidazole ring. This site was chosen, not for any strategic reasons, but because a synthetic route was readily available to carry out that particular transformation.

As hoped, the resulting compound, thiaburimamide (Fig. 13.27), had a significantly lower pK_a of 6.25 and was found to have enhanced antagonistic activity. This result supported the theory that a reduction in the proportion of ionized tautomer was beneficial to receptor binding and activity.

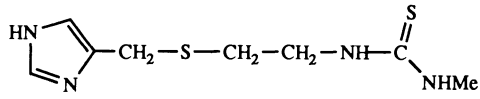


Fig. 13.27 Thiaburimamide.

Thiaburimamide had been synthesized in order to favour the un-ionized imidazole ring over the ionized ring. However, as we have seen, there are two possible un-ionized tautomers. The next question is whether either of these are preferred for receptor binding.

Let us return to histamine. If one of the un-ionized tautomers is preferred over the other in histamine, then it would be reasonable to assume that this is the favoured tautomer for receptor binding. The preferred tautomer for histamine is tautomer I (Fig. 13.26).

Why is tautomer I favoured? The answer lies in the fact that the side-chain on histamine is electron withdrawing. This electron withdrawing effect on the imidazole ring is inductive and therefore the strength of the effect will decrease with distance round the ring. This implies that the nitrogen atom on the imidazole ring closest to the side-chain ($N\pi$) will experience a greater electron withdrawing effect than the one further away ($N\tau$). As a result, the closer nitrogen is less basic, which in turn means that it is less likely to bond to hydrogen (tautomer I).

Since the side-chain in thiaburimamide is electron withdrawing, then it too will favour tautomer I.

It was now argued that this tautomer could be further enhanced if an electron **donating** group was placed at position 4 in the ring. At this position, the inductive effect would be felt most at the neighbouring nitrogen (N^{τ}), further enhancing its basic character and increasing the population of tautomer I. However, it was important to choose a group which would not interfere with the normal receptor binding interaction. For example, a large substituent might be too bulky and prevent the analogue fitting the receptor. A methyl group was chosen since it was known that 4-methylhistamine was an agonist and also highly selective for the H2 receptor (see Section 13.6.).

The compound obtained was metiamide (Fig. 13.28) which was found to have enhanced activity as an antagonist, supporting the previous theory.

It is interesting to note that the above effect outweighs an undesirable rise in pK_a. By adding an electron donating methyl group, there has been a rise in the pK_a of the imidazole ring to 6.80 compared to 6.25 for thiaburimamide. (Coincidentally, this is

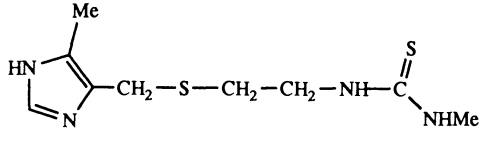


Fig. 13.28 Metiamide.

the same pK_a as for imidazole itself, which shows that the electronic effects of the methyl group and the side-chain are cancelling each other out as far as pK_a is concerned.) A pK_a of 6.80 means that 20 per cent of metiamide is ionized in the imidazole ring. However, this is still significantly lower than the corresponding 40 per cent for burimamide.

Compared to burimamide, the percentage of ionized imidazole ring has been lowered in metiamide and the ratio of the two possible un-ionized imidazole tautomers reversed. The fact that activity is increased with respect to **thiaburimamide** suggests that the increase in the population of tautomer (I) outweighs the increase in population of the ionized tautomer (III).

4-Methylburimamide (Fig. 13.29) was also synthesized for comparison. Here, the introduction of the 4-methyl group does not lead to an increase in activity. The pK_a is increased to 7.80, resulting in the population of ionized imidazole ring rising to 72 per cent. This demonstrates how important it is to rationalize structural changes. Adding the 4-methyl group to thiaburimamide is advantageous, but adding it to burimamide is not.

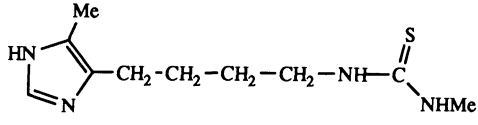


Fig. 13.29 4-Methylburimamide.

The design and synthesis of metiamide followed a rational approach aimed at favouring one specific tautomer. Such a study is known as a dynamic structure–activity analysis.

Strangely enough, it has since transpired that the improvement in antagonism may have resulted from conformational effects. X-ray crystallography studies have indicated that the longer thioether linkage in the chain increases the flexibility of the side-chain and that the 4-methyl substituent in the imidazole ring may help to orientate the imidazole ring correctly for receptor binding. It is significant that the oxygen analogue oxaburimamide (Fig. 13.30) is less potent than burimamide despite the fact that the electron withdrawing effect of the oxygen-containing chain on the ring is similar to the sulfur-containing chain. The bond lengths and angles of the ether link are similar to the methylene unit and in this respect it is a better isostere than sulfur. However, the oxygen atom is substantially smaller. It is also significantly more basic and more hydrophilic than either sulfur or methylene. Oxaburimamide's lower activity might be due to a variety of reasons. For example, the oxygen may not allow the same flexibility permitted by the sulfur atom. Alternatively, the oxygen may be involved in a hydrogen bonding interaction either with the receptor or with its own imidazole ring, resulting in a change in receptor binding interaction.

Metiamide is ten times more active than burimamide and showed promise as an

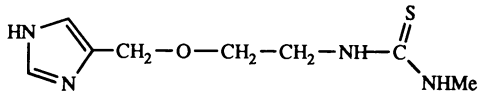


Fig. 13.30 Oxaburimamide.

antiulcer agent. Unfortunately, a number of patients suffered from kidney damage and granulocytopenia—a condition which results in the reduction of circulating white blood cells and which makes patients susceptible to infection. Further developments were now required to find an improved drug lacking these side-effects.

13.10 *Development of cimetidine*

It was proposed that metiamide's side-effects were associated with the thiourea group—a group which is not particularly common in the body's biochemistry. Therefore, consideration was given to replacing this group with a group which was similar in property but would be more acceptable in a biochemical context. The urea analogue (Fig. 13.31) was tried, but found to be less active. The guanidine analogue (Fig. 13.32) was also less active, but it was interesting to note that this compound had no agonist activity. This contrasts with the three-carbon bridged guanidine (Fig. 13.18) which we have already seen is a partial agonist. Therefore, the guanidine analogue (Fig 13.32) was the first example of a guanidine having pure antagonist activity.

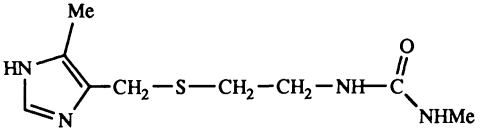


Fig. 13.31 Urea analogue.

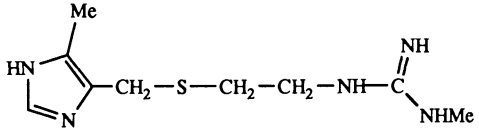


Fig. 13.32 Guanidine analogue.

One possible explanation for this is that the longer four-unit chain extends the guanidine binding group beyond the reach of the agonist binding site (Fig. 13.33), whereas the shorter three-unit chain still allows binding to both agonist and antagonist site (Fig. 13.34).

The antagonist activity for the guanidine analogue (Fig. 13.32) is weak, but it was decided to look more closely at this compound since it was thought that the guanidine unit would be less likely to have toxic side-effects than the thiourea. This is a reasonable assumption since the guanidine unit is present naturally in the amino acid arginine (Fig. 13.35). The problem now was to retain the guanidine unit, but to increase activity. It seemed likely that the low activity was due to the fact that the basic guanidine group would be ionized at pH 7.4. The problem was how to make this group neutral—no easy task, considering that guanidine is one of the strongest bases in organic chemistry.

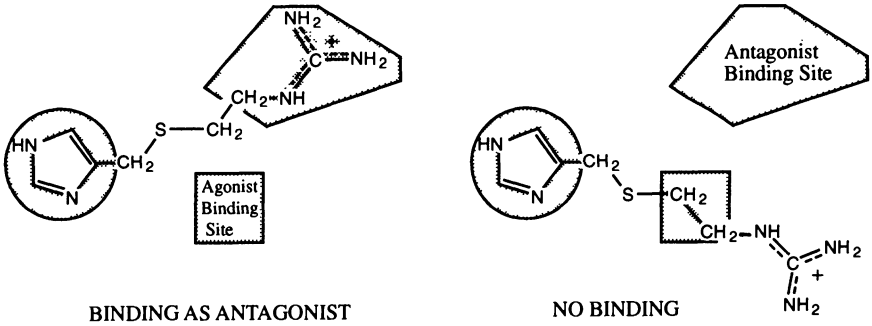


Fig. 13.33 Four-carbon unit chain.

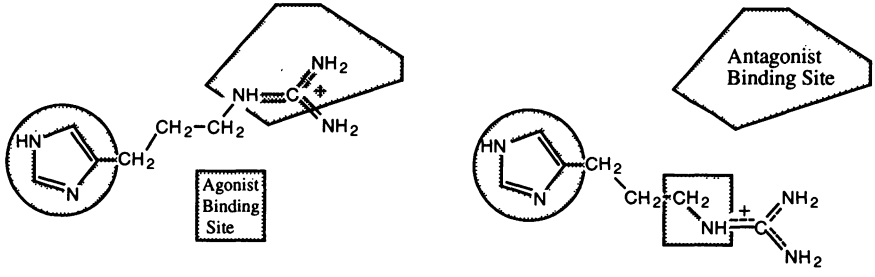


Fig. 13.34 Three-carbon unit chain.

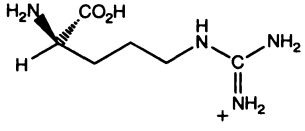


Fig. 13.35 Arginine.

Nevertheless, a search of the literature revealed a useful study on the ionization of monosubstituted guanidines (Fig. 13.36). A comparison of the pK_a values of these compounds with the inductive substituent constants σ_I for the substituents X gave a straight line as shown in Fig. 13.37, showing that pK_a is inversely proportional to the electron withdrawing power of the substituent. Thus, strongly electron withdrawing substituents make the guanidine group less basic and less ionized. The nitro and cyano groups are particularly strong electron withdrawing groups. The ionization constants for cyanoguanidine and nitroguanidine are 0.4 and 0.9 respectively (Fig. 13.37)—similar values to the ionization constant for thiourea itself (−1.2).

Both the nitroguanidine and cyanoguanidine analogues of metiamide were synthesized and found to have comparable antagonist activities to metiamide. The

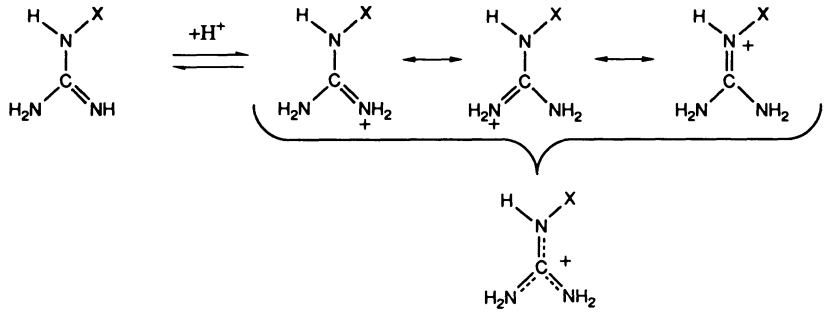


Fig. 13.36 Ionization of monosubstituted guanidines.

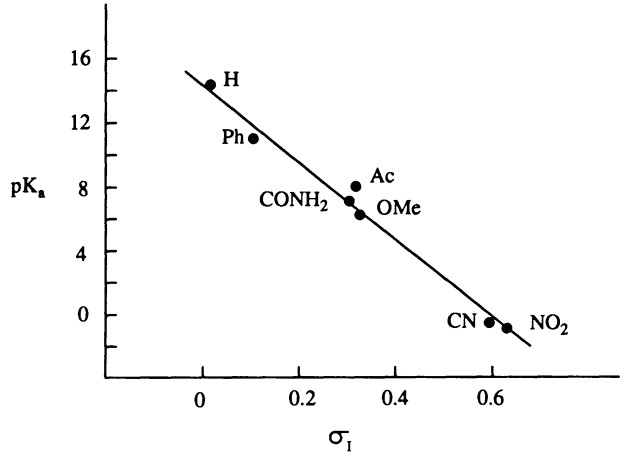


Fig. 13.37 PK_a vs. inductive substituent constants (σ_I) for the substituents X in Fig. 13.36.

cyanoguanidine analogue (cimetidine (Fig. 13.1)) was the more potent analogue and was chosen for clinical studies.

13.11 *Cimetidine*

13.11.1 Biological activity of cimetidine

Cimetidine inhibits H2 receptors and thus inhibits gastric acid release. The drug does not show the toxic side-effects observed for metiamide and has been shown to be slightly more active. It has also been found to inhibit pentagastrin (Fig. 13.38) from stimulating release of gastric acid. Pentagastrin is an analogue of gastrin and the fact that cimetidine blocks its stimulatory activity suggests some relationship between histamine and gastrin in the release of gastric acid.

Cimetidine was first marketed in the UK in 1976 and for several years was the

Fig. 13.38 Pentagastrin. N-t-BOC-ß-Ala-Trp-Met-Asp-Phe-NH_2

world's biggest selling prescription product until it was pushed into second place in 1988 by ranitidine (Section 13.13.).

13.11.2 Structure and activity of cimetidine

The finding that metiamide and cimetidine are both good H2 antagonists of similar activity shows that the cyanoguanidine group is a good bioisostere for the thiourea group.

This is despite the fact that three tautomeric forms (Fig. 13.39) are possible for the guanidine group compared to only one for the isothiourea group. In fact, this is more apparent than real, since the imino tautomer (II) is the preferred tautomeric form for the guanidine unit. Tautomer II is favoured since the cyano group has a stronger electron withdrawing effect on the neighbouring nitrogen compared to the two nitrogens further away. This makes the neighbouring nitrogen less basic and therefore less likely to be protonated.

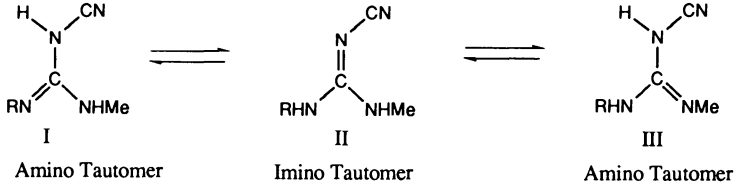


Fig. 13.39 Three tautomeric forms of guanidine unit.

Since tautomer II is favoured, the guanidine group does in fact bear a close structural similarity to the thiourea group. Both groups have a planar pi electron system with similar geometries (equal C–N distances and angles). They are polar and hydrophilic with high dipole moments and low partition coefficients. They are weakly basic and also weakly acidic such that they are un-ionized at pH 7.4.

13.11.3 Metabolism of cimetidine

It is important to study the metabolism of any new drug in case the metabolites have biological activity in their own right. Any such activity might lead to side-effects. Alternatively, a metabolite might have enhanced activity of the type desired and give clues to further development.

Cimetidine itself is metabolically stable and is excreted largely unchanged. The only metabolites which have been identified are due to oxidation of the sulfur link (Fig. 13.40) or oxidation of the ring methyl group (Fig. 13.41).

It has been found that cimetidine inhibits the P-450 cytochrome oxidase system in the liver. This is an important enzyme system in the metabolism of drugs and care must be taken if other drugs are being taken at the same time as cimetidine, since

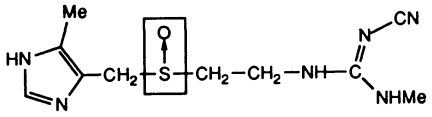


Fig. 13.40 Oxidation of the sulfur link in cimetidine.

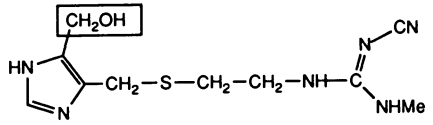


Fig. 13.41 Oxidation of the ring methyl group in cimetidine.

cimetidine may inhibit the metabolism of these drugs, leading to higher blood levels and toxic side-effects. In particular, care must be taken when cimetidine is taken with drugs such as diazepam, lidocaine, warfarin, or theophylline.

13.12 *Further studies—cimetidine analogues*

13.12.1 Conformational isomers

A study of the various stable conformations of the guanidine group in cimetidine led to a rethink of the type of bonding which might be taking place at the antagonist site. Up until this point, the favoured theory had been a bidentate hydrogen interaction as shown in Fig. 13.17.

In order to achieve this kind of bonding, the guanidine group in cimetidine would have to adopt the *Z,Z* conformation shown in Fig. 13.42.

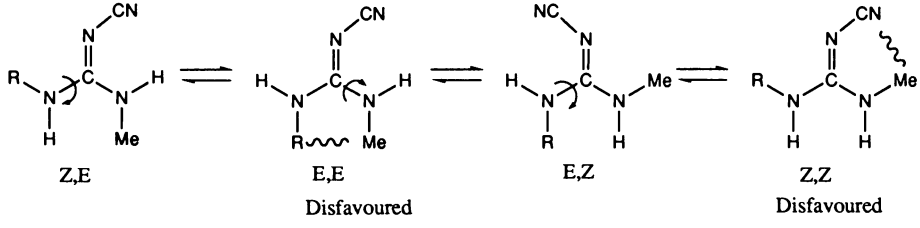


Fig. 13.42 Conformations of the guanidine group in cimetidine.

However, X-ray and NMR studies have shown that cimetidine exists as an equilibrium mixture of the *E,Z* and *Z,E* conformations. The *Z,Z* form is not favoured since the cyano group is forced too close to the *N*-methyl group. If either the *E,Z* or *Z,E* form is the active conformation, then it implies that the chelation type of hydrogen bonding described previously is not essential. An alternative possibility is that the guanidine unit is hydrogen bonding to two distinct hydrogen bonding sites rather than to a single carboxylate group (Fig. 13.43).

Further support for this theory is provided by the weak activity observed for the urea analogue (Fig. 13.32). This compound is known to prefer the *Z,Z* conformation over the *Z,E* or *E,Z* and would therefore be unable to bind to both hydrogen bonding sites.

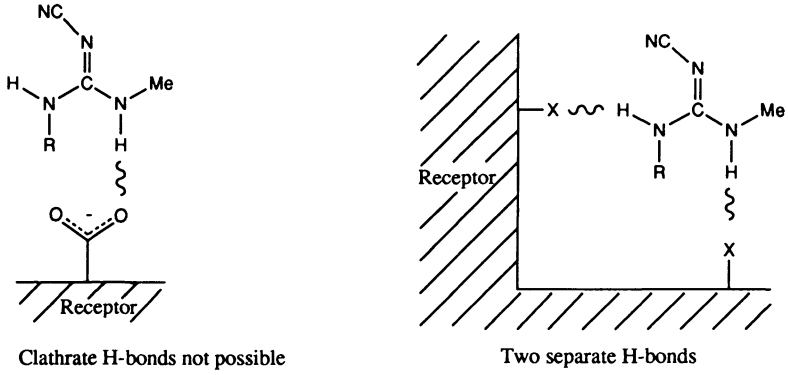


Fig. 13.43 Alternative theory for cimetidine bonding at the agonist site.

If this bonding theory is correct and the active conformation is the *E,Z* or *Z,E* form, then restricting the group to adopt one or other of these forms may lead to more active compounds and an identification of the active conformation. This can be achieved by incorporating part of the guanidine unit within a ring—a strategy of rigidification.

For example, the nitropyrrole derivative (Fig. 13.44) has been shown to be the strongest antagonist in the cimetidine series, implying that the *E,Z* conformation is the active conformation.

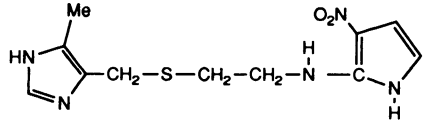


Fig. 13.44 Nitropyrrole derivative of cimetidine.

The isocytosine ring (Fig. 13.45) has also been used to 'lock' the guanidine group, limiting the number of conformations available. The ring allows further substitution and development as seen below (Section 13.12.2.).

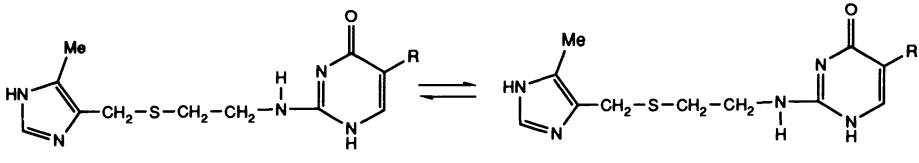


Fig. 13.45 Isocytosine ring.

13.12.2 Desolvation

Development of oxmetidine

It has already been stated that the guanidine and thiourea groups, used so successfully in the development of H2 antagonists, are polar and hydrophilic. This implies that

they are likely to be highly solvated (i.e. surrounded by a 'water coat'). Before hydrogen bonding can take place to the receptor, this 'water coat' has to be removed. The more solvated the group, the more difficult that will be.

One possible reason for the low activity of the urea derivative (Fig. 13.32) has already been described above. Another possible reason could be the fact that the urea group is more hydrophilic than thiourea or cyanoguanidine groups and therefore more highly solvated. The difficulty in desolvating the urea group might explain why the urea analogue has a lower activity than cimetidine, despite having a lower partition coefficient and greater water solubility.

Leading on from this, if the ease of desolvation is a factor in antagonist activity, then reducing the solvation of the polar group should increase activity. One way of achieving this would be to increase the hydrophobic character of the polar binding group.

A study was carried out on a range of cimetidine analogues containing different planar aminal systems (Z) (Fig. 13.46) to see whether there was any relationship between antagonist activity and the hydrophobic character of the aminal system (HZ).

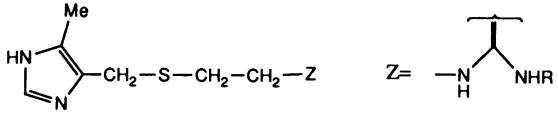


Fig. 13.46 Cimetidine analogue with planar animal system (Z).

This study showed that antagonist activity was proportional to the hydrophobicity of the aminal unit Z (Fig. 13.47) and supported the desolvation theory. The relationship could be quantified as follows:

$$\log(\text{activity}) = 2.0 \log P + 7.4$$

Further studies on hydrophobicity were carried out by adding hydrophobic substituents to the isocytosine analogue (Fig. 13.45). These studies showed that there was an optimum hydrophobicity for activity corresponding to the equivalent of a butyl or pentyl substituent. A benzyl substituent was particularly good for activity, but proved to have toxic side-effects. These side-effects could be reduced by adding alkoxy substituents to the aromatic ring and this led to the synthesis of oxmetidine (Fig. 13.48) which had enhanced activity over cimetidine. Oxmetidine was considered for clinical use, but was eventually withdrawn since it still retained undesirable side-effects.

The development of the nitroketeneaminal binding group

As we have seen, antagonist activity increases with the hydrophobicity of the polar binding group. It was therefore decided to see what would happen if the polar imino

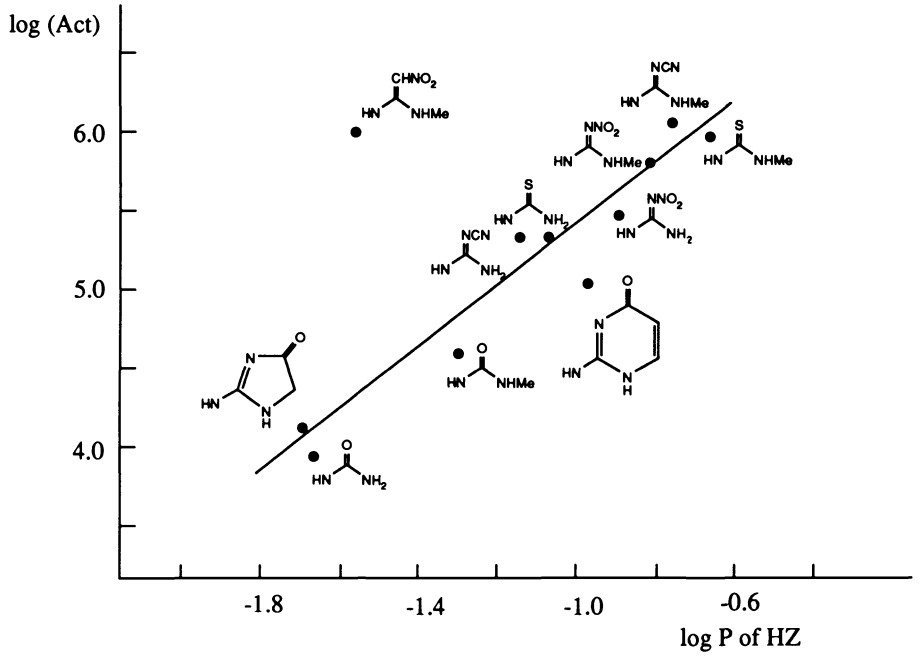


Fig. 13.47 Antagonist activity is proportional to the hydrophobicity of the animal unit Z.

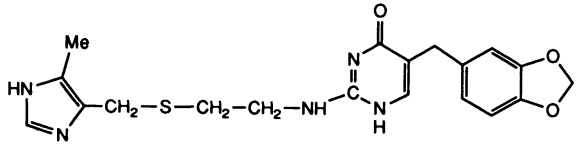


Fig. 13.48 Oxmetidine.

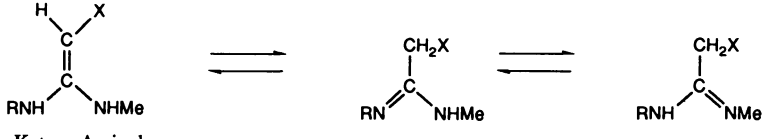


Fig. 13.49

nitrogen of cimetidine was replaced with a non-polar carbon atom. This would result in a keteneaminal group as shown in Fig. 13.49. Unfortunately, keteneaminals are more likely to exist as their amidine tautomers unless a strongly electronegative group (e.g. NO_2) is attached to the carbon atom.

Therefore, a nitroketeneaminal group was used to give the structure shown in Fig. 13.50. Surprisingly, there was no great improvement in activity, but when the structure was studied in detail, it was discovered that it was far more hydrophilic then

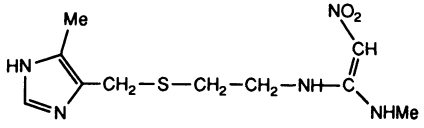


Fig. 13.50 Cimetidine analogue with a nitroketeneaminal group.

expected. This explained why the activity had not increased, but it highlighted a different puzzle. The compound was *too* active. Based on its hydrophilicity, it should have been a weak antagonist (Fig. 13.47).

It was clear that this compound did not fit the pattern followed by previous compounds since the antagonist activity was 30 times higher than would have been predicted by the equation above. Nor was the nitroketeneaminal the only analogue to deviate from the normal pattern. The imidazolinone analogue (Fig. 13.51), which is relatively hydrophobic, had a much lower activity than would have been predicted from the equation.

Findings like these are particularly exciting since any deviation from the normal pattern suggests that some other factor is at work which may give a clue to future development.

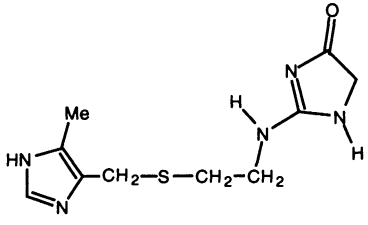


Fig. 13.51 Imidazolinone analogue.

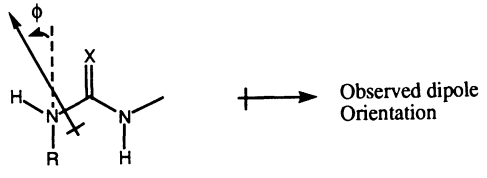
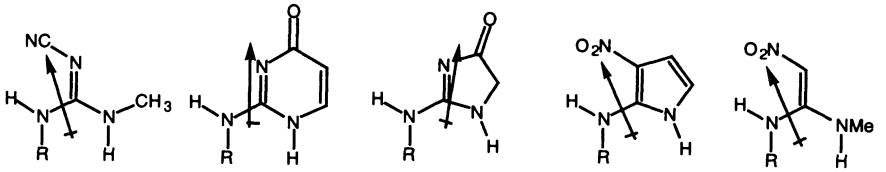


Fig. 13.52 Orientation of dipole moment.

In this case, it was concluded that the polarity of the group might be important in some way. In particular, the **orientation** of the dipole moment appeared to be crucial. In Fig. 13.52, the orientation of the dipole moment is defined by ϕ—the angle between the dipole moment and the NR bond. The cyanoguanidine, nitroketeneaminal, and nitropyrrole groups all have high antagonist activity and have dipole moment orientations of 13, 33, and 27° respectively (Fig. 13.53). The isocytosine and imidazolinone groups result in lower activity and have dipole orientations of 2 and −6°, respectively. The strength of the dipole moment (μ) does not appear to be crucial.

Why should the orientation of a dipole moment be important? One possible explanation is as follows. As the drug approaches the receptor, its dipole interacts with a dipole on the receptor surface such that the dipole moments are aligned. This orientates the drug in a specific way before hydrogen bonding takes place and will determine how strong the subsequent hydrogen bonding will be (Fig. 13.54). If the



ϕ = 13
μ = 13.1

ϕ = 2
μ = 13.1

ϕ = -6
μ = 16.7

ϕ = 27
μ = 14.2

ϕ = 33
μ = 15.1

Fig. 13.53 Dipole moments of various antagonistic groups.

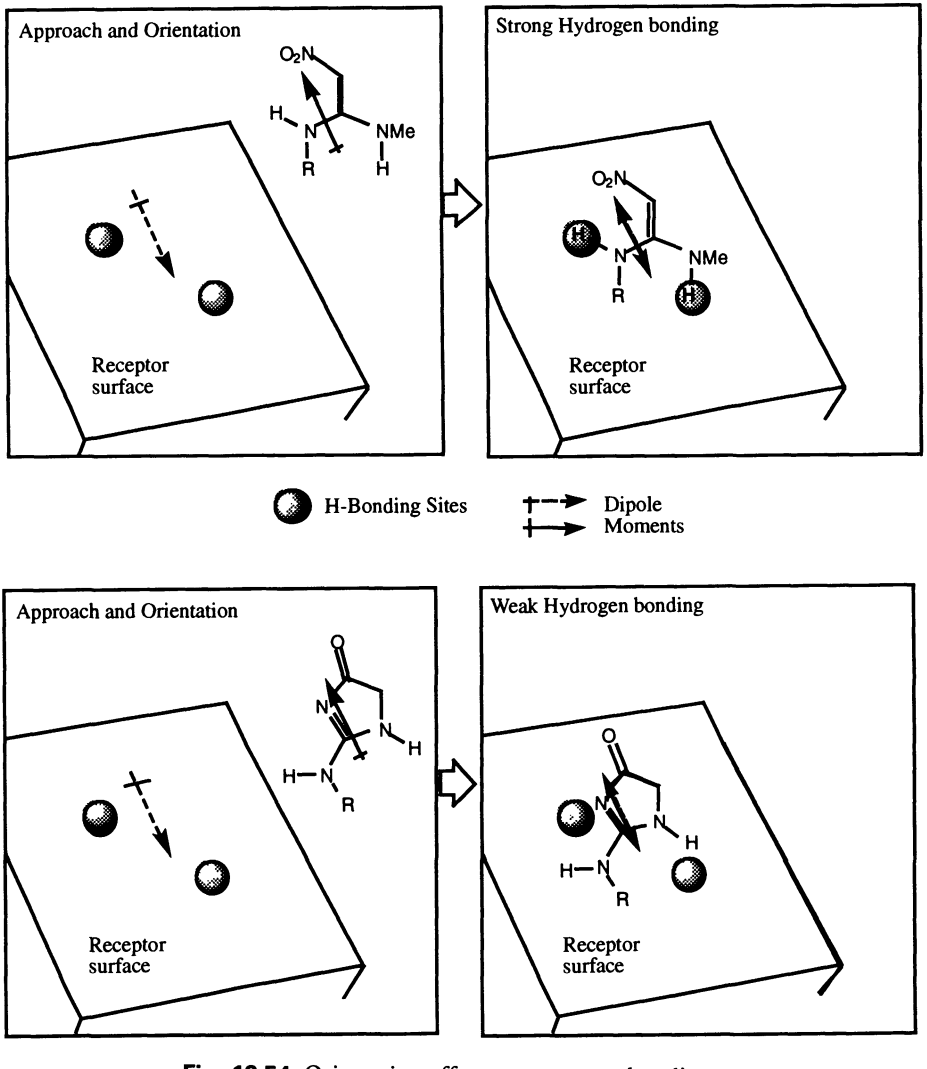


Fig. 13.54 Orientation effects on receptor bonding.

dipole moment is correctly orientated as in the keteneaminal analogue, the group will be correctly positioned for strong hydrogen bonding and high activity will result. If the orientation is wrong as in the imidazolinone analogue, then the bonding is less efficient and activity is lost.

QSAR studies were carried out to determine what the optimum angle ϕ should be for activity. This resulted in an ideal angle for ϕ of 30°. A correlation was worked out between the dipole moment orientation, partition coefficient, and activity as follows:

$$\log A = 9.12 \cos \theta + 0.6 \log P - 2.71$$

where A is the antagonist activity, P is the partition coefficient, and θ is the deviation in angle of the dipole moment from the ideal orientation of 30° (Fig. 13.55).

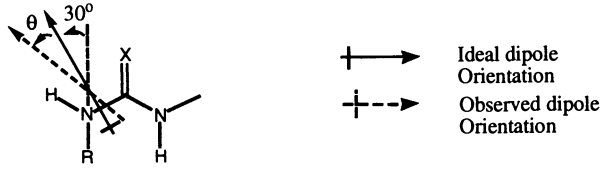


Fig. 13.55

The equation shows that activity increases with increasing hydrophobicity (P). The cos θ term shows that activity drops if the orientation of the dipole moment varies from the ideal angle of 30°. At the ideal angle, θ is 0° and cos θ is 1. If the orientation of the dipole moment deviates from 30°, then cos θ will be a fraction and will lower the calculated activity. The nitroketeneaminal group did not result in a more powerful cimetidine analogue, but we shall see it appearing again in ranitidine (Section 13.13.).

13.13 *Variation of the imidazole ring—ranitidine*

Further studies on cimetidine analogues showed that the imidazole ring could be replaced with other nitrogen-containing heterocyclic rings.

However, Glaxo moved one step further by showing that the imidazole ring could be replaced with a furan ring bearing a nitrogen-containing substituent. This led to the introduction of ranitidine (Zantac) (Fig. 13.56).

Ranitidine has fewer side-effects than cimetidine, lasts longer, and is ten times more active. Structure–activity results for ranitidine include the following:

- The nitroketeneaminal group is optimum for activity, but can be replaced with other planar pi systems capable of hydrogen bonding.

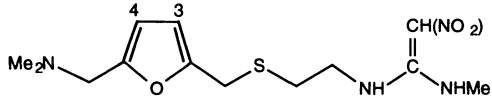


Fig. 13.56 Ranitidine.

- Replacing the sulfur atom with a methylene unit leads to a drop in activity.
- Placing the sulfur next to the ring lowers activity.
- Replacing the furan ring with more hydrophobic rings such as phenyl or thiophene reduces activity.
- 2,5-Disubstitution is the best substitution pattern for the furan ring.
- Substitution on the dimethylamino group can be varied, showing that the basicity and hydrophobicity of this group are not crucial to activity.
- Methyl substitution at carbon-3 of the furan ring eliminates activity, whereas the equivalent substitution in the imidazole series increases activity.
- Methyl substitution at carbon-4 of the furan ring increases activity.

The latter two results imply that the heterocyclic rings for cimetidine and ranitidine are not interacting in the same way with the H2 receptor. This is supported by the fact that a corresponding dimethylaminomethylene group attached to cimetidine leads to a drop in activity.

Ranitidine was introduced to the market in 1981 and by 1988 was the world's biggest selling prescription drug.

13.14 *Famotidine and nizatidine*

During 1985/87 two new antiulcer drugs were introduced to the market—famotidine and nizatidine.

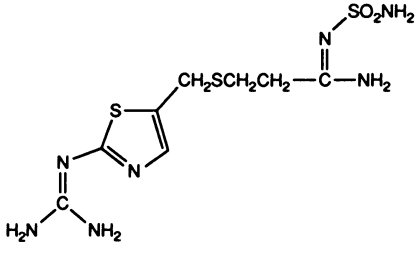


Fig. 13.57 Famotidine.

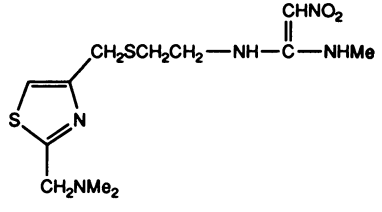


Fig. 13.58 Nizatidine.

Famotidine (Pepcid) (Fig. 13.57) is 30 times more active than cimetidine in vitro. The side-chain contains a sulfonylamidine group while the heterocyclic imidazole ring of cimetidine has been replaced with a 2-guanidinothiazole ring. Structure–activity studies gave the following results:

- The sulfonylamidine binding group is not essential and can be replaced with a variety of structures as long as they are planar, have a dipole moment, and are capable of interacting with the receptor by hydrogen bonding. A low pK_a is not

essential, which allows a larger variety of planar groups to be used than is possible for cimetidine.

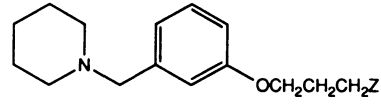
- Activity is optimum for a chain length of four or five units.
- Replacement of sulfur with a CH_2 group *increases* activity.
- Modification of the chain is possible with, for example, inclusion of an aromatic ring.
- A methyl substituent *ortho* to the chain leads to a drop in activity (unlike the cimetidine series).
- Three of the four hydrogens in the two NH_2 groups are required for activity.

There are several results here which are markedly different from cimetidine, implying that famotidine and cimetidine are not interacting in the same way with the H2 receptor. Further evidence for this is the fact that guanidine substitution at the equivalent position of cimetidine analogues leads to very low activity.

Nizatidine (Fig. 13.58) was introduced into the UK in 1987 by the Lilly Corporation and is equipotent with ranitidine. The furan ring in ranitidine is replaced with a thiazole ring.

13.15 *H2 antagonists with prolonged activity*

There is presently a need for longer lasting antiulcer agents which require once daily doses. Glaxo carried out further development on ranitidine by placing the oxygen of the furan ring exocyclic to a phenyl ring and replacing the dimethylamino group with a piperidine ring to give a series of novel structures (Fig. 13.59).

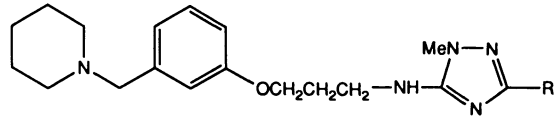


Z= planar and polar H bonding group

Fig. 13.59 Long lasting anti-ulcer agents.

The most promising of these compounds were lamitidine and loxtidine (Fig. 13.60) which were five to ten times more potent than ranitidine and three times longer lasting.

Unfortunately, these compounds showed toxicity in long-term animal studies with



R= NH_2 LAMITIDINE
R= CH_2OH LOXTIDINE

Fig. 13.60 Lamitidine and loxtidine.

the possibility that they caused gastric cancer, and they were subsequently withdrawn from clinical study. However, the relevance of these results has been disputed.

13.16 *Comparison of H1 and H2 antagonists*

The structures of the H2 antagonists are markedly different to the classical H1 antagonists and so there can be little surprise that these original antihistamines failed to antagonize the H2 receptor.

H1 antagonists, like H1 agonists, possess an ionic amino group at the end of a flexible chain. Unlike the agonists, they possess two aryl or heteroaryl rings in place of the imidazole ring. (Fig. 13.61). Because of the aryl rings, H1 antagonists are hydrophobic molecules having high partition coefficients.

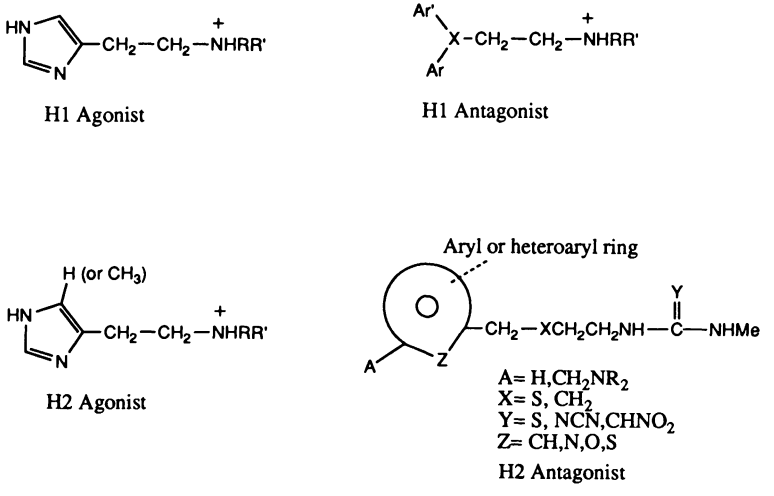


Fig. 13.61 Comparison between H1 and H2 agonists and antagonists.

In contrast, H2 antagonists are polar, hydrophilic molecules having high dipole moments and low partition coefficients. At the end of the flexible chain they have a polar, pi electron system which is weakly amphoteric and un-ionized at pH 7.4. This binding group appears to be the key feature leading to antagonism of H2 receptors (Fig. 13.61). The five-membered heterocycle generally contains a nitrogen atom or, in the case of furan or phenyl, a nitrogen-containing side-chain. The hydrophilic character of H2 antagonists helps to explain why H2 antagonists are less likely to have the CNS side-effects often associated with H1 antagonists.

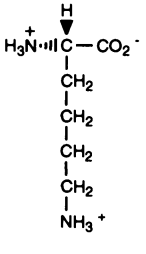
13.17 *The H2 receptor and H2 antagonists*

H2 receptors are present in a variety of organs and tissues, but their main role is in acid secretion. As a result, H2 antagonists are remarkably safe and mostly free of side-

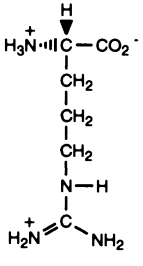
effects. The four most used agents on the market are cimetidine, ranitidine, famotidine, and nizatidine. They inhibit all aspects of gastric secretion and are rapidly absorbed from the gastrointestinal tract with half-lives of 1–2 h. About 80 per cent of ulcers are healed after 4–6 weeks.

Attention must be given to possible drug interactions when using cimetidine due to inhibition of drug metabolism (Section 13.11.). The other three H2 antagonists mentioned do not inhibit the P-450 cytochrome oxidase system and are less prone to such interactions.

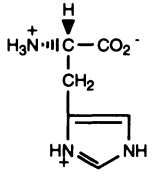
IONISED



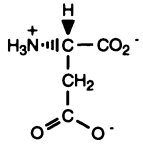
Lysine
(Lys)



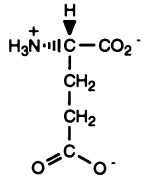
Arginine
(Arg)



Histidine
(His)



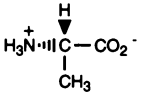
Aspartate
(Asp)



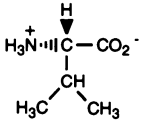
Glutamate
(Glu)

Appendix 1 ▪ Essential amino acids

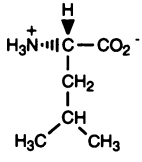
NON POLAR
(hydrophobic)



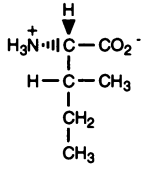
Alanine
(Ala)



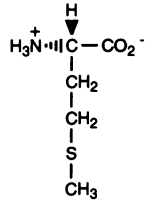
Valine
(Val)



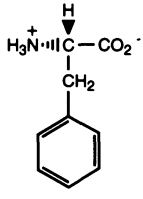
Leucine
(Leu)



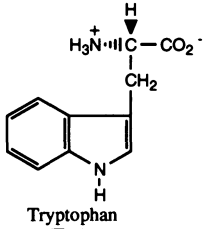
Isoleucine
(Ile)



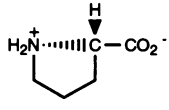
Methionine
(Met)



Phenylalanine
(Phe)

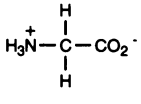


Tryptophan
(Trp)

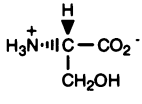


Proline
(Pro)

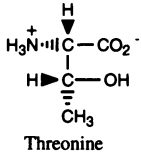
POLAR



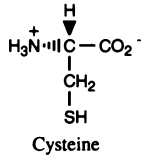
Glycine
(Gly)



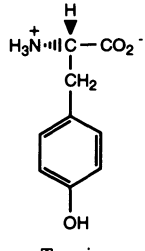
Serine
(Ser)



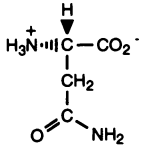
Threonine
(Thr)



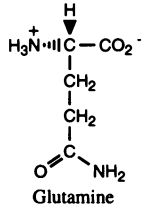
Cysteine
(Cys)



Tyrosine
(Tyr)



Asparagine
(Asn)



Glutamine
(Gln)

Appendix 2 ▪ The action of nerves

The structure of a typical nerve is shown in Fig. A2.1. The nucleus of the cell is found in the large cell body situated at one end of the nerve cell. Small arms (dendrites) radiate from the cell body and receive messages from other nerves. These messages either stimulate or destimulate the nerve. The cell body 'collects' the sum total of these messages.

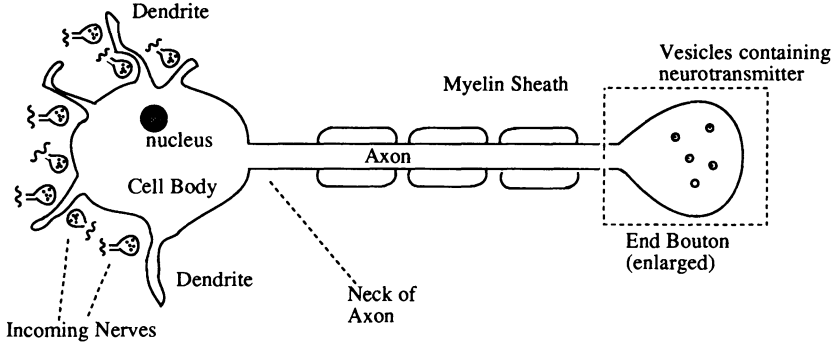


Fig. A2.1 Structure of a typical nerve cell.

It is worth emphasizing the point that the cell body of a nerve receives messages not just from one other nerve, but from a range of different nerves. These pass on different messages (neurotransmitters). Therefore, a message received from a single nerve is unlikely to stimulate a nerve signal by itself unless other nerves are acting in sympathy.

Assuming that the overall stimulation is great enough, an electrical signal is 'fired' down the length of the nerve (the axon). This axon is 'padded' with sheaths of lipid (myelin sheaths) which act to insulate the signal as it passes down the axon.

The axon leads to a knob-shaped swelling (synaptic button) if the nerve is communicating with another nerve. Alternatively, if the nerve is communicating with a muscle cell, the axon leads to what is known as a neuromuscular endplate, where the nerve cell has spread itself like an amoeba over an area of the muscle cell.

Within the synaptic button or neuromuscular endplate there are small globules

(vesicles) containing the neurotransmitter chemical. When a signal is received from the axon, the vesicles merge with the cell membrane and release their neurotransmitter into the gap between the nerve and the target cell (synaptic gap). The neurotransmitter binds to the receptor as described in Chapter 5 and passes on its message. Once the message has been received, the neurotransmitter leaves the receptor and is either broken down enzymatically (e.g. acetylcholine) or taken up intact by the nerve cell (e.g. noradrenaline). Either way, the neurotransmitter is removed from the synaptic gap and is unable to bind with its receptor a second time.

To date, we have talked about nerves 'firing' and the generation of 'electrical signals' without really considering the mechanism of these processes. The secret behind nerve transmission lies in the movement of ions across cell membranes, but there is an important difference in what happens in the cell body of a nerve compared to the axon. We shall consider what happens in the cell body first.

All cells contain sodium, potassium, calcium, and chloride ions and it is found that the concentration of these ions is different inside the cell compared to the outside. The concentration of potassium inside the cell is larger than the surrounding medium, whereas the concentration of sodium and chloride ions is smaller. Thus, a concentration gradient exists across the membrane.

Potassium is able to move down its concentration gradient (i.e. out of the cell) since it can pass through the potassium ion channels (Fig. A2.2). However, if potassium

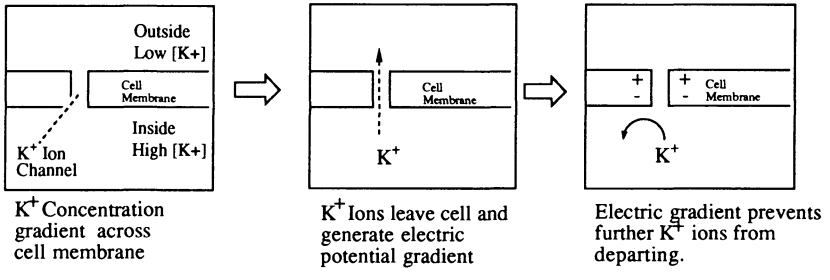


Fig. A2.2 Generation of electric potential across a cell membrane.

can move out of the cell, why does the potassium concentration inside the cell not fall to equal that of the outside? The answer lies in the fact that potassium is a positively charged ion and as it leaves the cell an electric potential is set up across the cell membrane. This would not happen if a negatively charged counterion could leave with the potassium ion. However, the counterions in question are large proteins which cannot pass through the cell membrane. As a result, a few potassium ions are able to escape down the ion channels out of the cell and an electric potential builds up across the cell membrane such that the inside of the cell membrane is more negative than the outside. This electric potential (50–80 mV) opposes and eventually prevents the flow of potassium ions

But what about the sodium ions? Could they flow into the cell along their concen-

tration gradient to balance the charged potassium ions which are departing? The answer is that they cannot because they are too big for the potassium ion channels. This appears to be a strange argument since sodium ions are smaller than potassium ions. However, it has to be remembered that we are dealing with an aqueous environment where the ions are solvated (i.e. they have a 'coat' of water molecules). Sodium, being a smaller ion than potassium, has a greater localization of charge and is able to bind its solvating water molecules more strongly. As a result, sodium along with its water coat is bigger than a potassium ion with or without its water coat.

Ion channels for sodium do exist and these channels are capable of removing the water coat around sodium and letting it through. However, the sodium ion channels are mostly closed when the nerve is in the resting state. As a result, the flow of sodium ions across the membrane is very small compared to potassium. Nevertheless, the presence of sodium ion channels is crucial to the transmission of a nerve signal.

To conclude, the movement of potassium across the cell membrane sets up an electric potential across the cell membrane which opposes this same flow. Charged protein structures are unable to move across the membrane, while sodium ions cross very slowly, and so an equilibrium is established. The cell is polarized and the electric potential at equilibrium is known as the resting potential.

The number of potassium ions required to establish that potential is of the order of

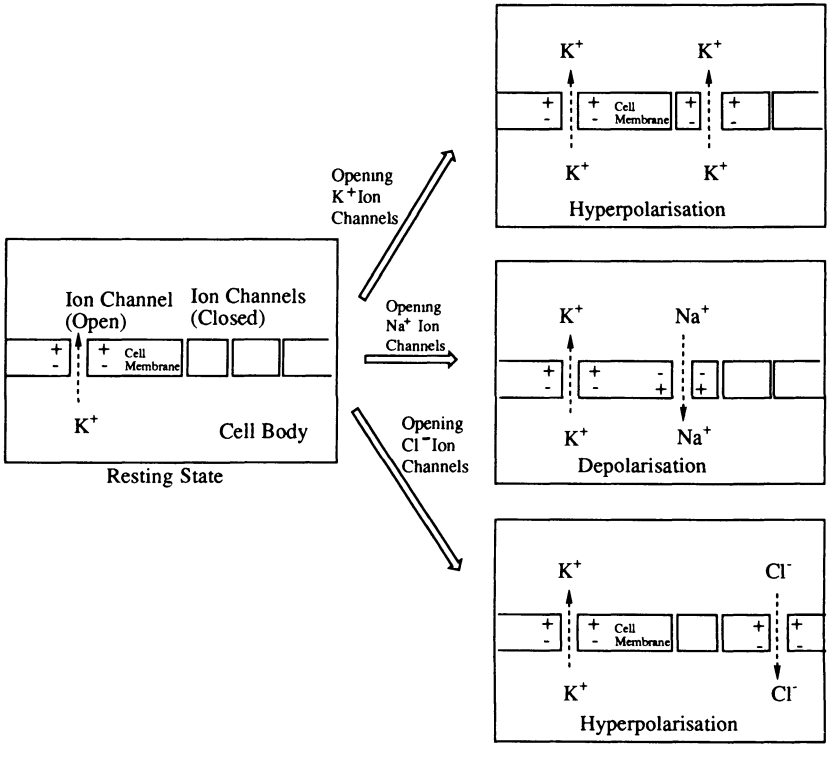


Fig. A2.3 Hyperpolarization and depolarization.

a few million compared to the several hundred billion present in the cell. Therefore the effect on concentration is negligible.

As mentioned above, potassium ions are able to flow out of potassium ion channels. However, not all of these channels are open in the resting state. What would happen if more were to open? The answer is that more potassium ions would flow out of the cell and the electric potential across the cell membrane would become more negative to counter this increased flow. This is known as hyperpolarization and the effect is to destimulate the nerve (Fig. A2.3).

Suppose instead that a few sodium ion channels were to open up. In this case, sodium ions would flow into the cell and as a result the electric potential would become less negative. This is known as depolarization and results in a stimulation of the nerve.

If chloride ions channels are opened, chloride ions flow into the cell and the cell membrane becomes hyperpolarized, destimulating the nerve.

Ion channels do not open or close by chance. They are controlled by the neurotransmitters released by communicating nerves. The neurotransmitters bind with their receptors and lead to the opening or closing of ion channels. For example, acetylcholine controls the sodium ion channel, whereas GABA and glycine control chloride ion channels. The resulting flow of ions leads to a localized hyperpolarization or depolarization in the area of the receptor. The cell body collects and sums all this information such that the neck of the axon experiences an overall depolarization or hyperpolarization depending on the sum total of the various excitatory or inhibitory signals received.

We shall now consider what happens in the axon of the nerve (Fig. A2.4). The cell membrane of the axon also has sodium and potassium ion channels but they are different in character from those in the cell body. The axon ion channels are not controlled by neurotransmitters, but by the electric potential of the cell membrane.

The sodium ion channels located at the junction of the nerve axon with the cell body are the crucial channels since they are the first channels to experience whether the cell body has been depolarized or hyperpolarized.

If the cell body is strongly depolarized then a signal is fired along the nerve. However, a specific threshold value has to be reached before this happens. If the depolarization from the cell body is weak, only a few sodium channels open up and the depolarization at the neck of the axon does not reach that threshold value. The sodium channels then reclose and no signal is sent.

With stronger depolarization, more sodium channels open up until the flow of sodium ions entering the axon becomes greater than the flow of potassium ions leaving it. This results in a rapid increase in depolarization, which in turn opens up more sodium channels, resulting in very strong depolarization at the neck of the axon. The flow of sodium ions into the cell increases dramatically, such that it is far greater than the flow of potassium ions out of the axon, and the electric potential across the membrane is reversed, such that it is positive inside the cell and negative outside the cell. This process lasts less than a millisecond before the sodium channels reclose and sodium permeability returns to its normal state. More potassium channels then open

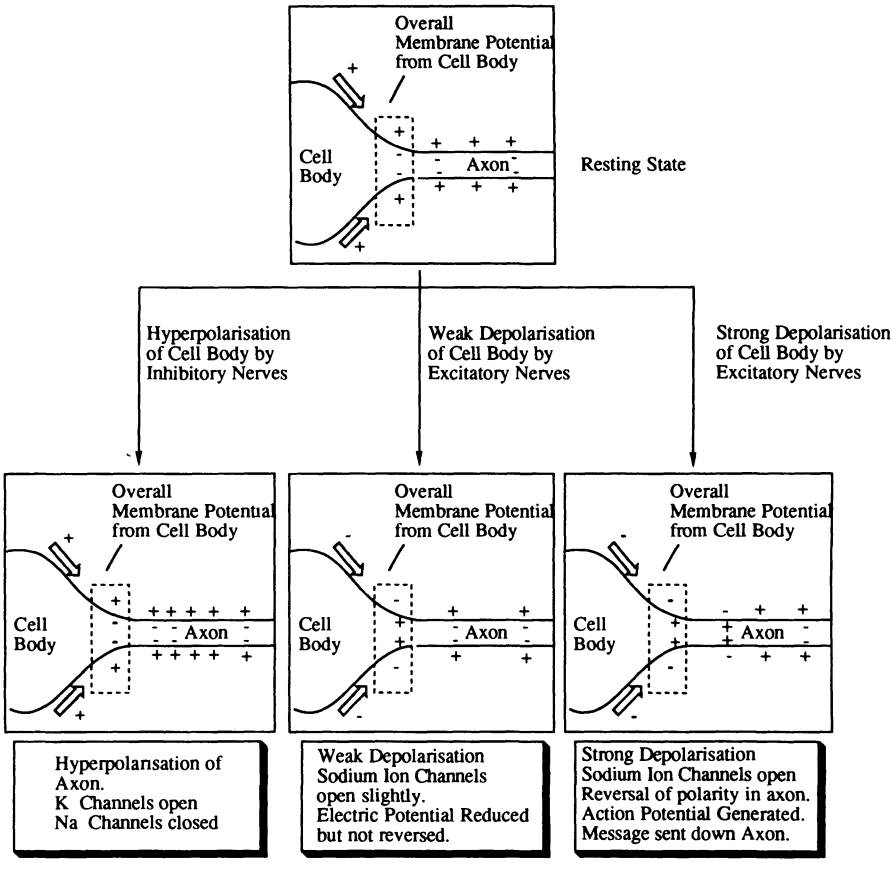


Fig. A2.4 Hyperpolarization and depolarization effects at the neck of the axon.

and permeability to potassium ions increases for a while to speed up the return to the resting state.

The process is known as an action potential and can only take place in the axon of the nerve. The cell membrane of the axon is said to be excitable, unlike the membrane of the cell body.

The important point to note is that once an action potential has fired at the neck of the axon, it has reversed the polarity of the membrane at the point. This in turn has an effect on the neighbouring area of the axon and depolarizes it beyond the critical threshold level. It too fires an action potential and so the process continues along the whole length of the axon (see Fig. A2.5).

The number of ions involved in this process is minute, such that the concentrations are unaffected.

Once the action potential reaches the synaptic button or the neuromuscular endplate, it causes an influx of calcium ions into the cell and an associated release of neurotransmitter into the synaptic gap. The mechanism of this is not well understood.

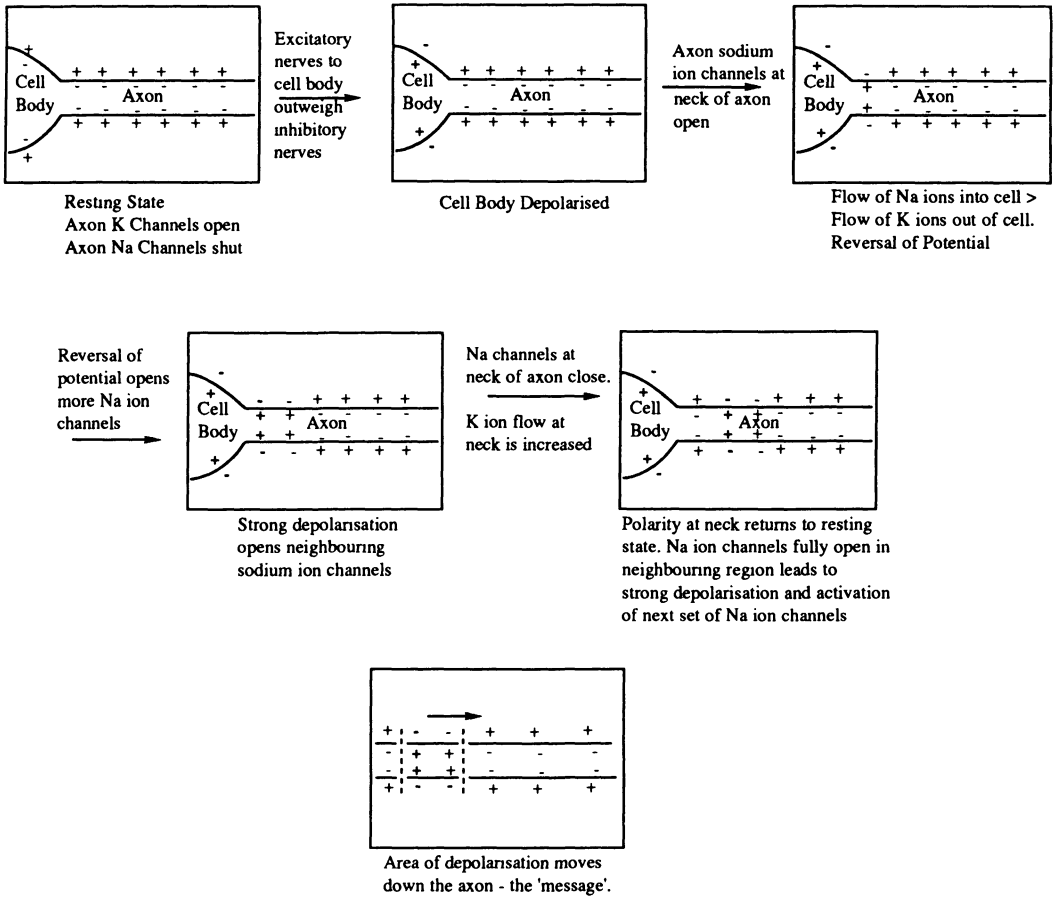


Fig. A2.5 Generation of an action potential.

Appendix 3 • Secondary messengers

In Chapter 5, we discussed how simple chemicals interact with receptor proteins to change the tertiary structure of these proteins. This change of structure can result in the opening or closing of ion channels, e.g. the acetylcholine receptor (see chapter 11).

Alternatively, the neurotransmitter/receptor interaction leads to the activation or deactivation of enzymes. In Chapter 5, this was represented as a direct process whereby the receptor and target enzyme are closely associated. In reality, the receptor and target enzyme are not directly associated and the interaction of receptor and neurotransmitter is the first step in a complex chain of events which involves several proteins and enzymes.

In fact, the receptor is not thought to be *directly* associated with any sort of protein, enzyme or otherwise. The sequence of events leading from the combination of receptor and ligand (the neurotransmitter) to the final activation of a target enzyme is thought to be as shown in Fig. A3.1.

The neurotransmitter binds to the receptor as already described, resulting in a change in the tertiary structure. This receptor/ligand complex is then thought to drift through the cell membrane until it meets a protein called the G_s protein. (The G_s protein acquires the label G since it binds the guanyl nucleotide GDP. The subscript S refers to the fact that the G_s protein stimulates the enzymic synthesis of a secondary messenger.) The G_s protein is situated at the inner surface of the cell membrane and is made up of three protein subunits, possibly linked by disulfide bridges.

The receptor ligand complex interacts in some way with the G_s protein and leads to the exchange of GDP with GTP (guanosine triphosphate). This in turn results in the fragmentation of the G_s protein giving off the $alpha_s$ subunit with GTP attached. The receptor ligand complex has now done its job and it dissociates.

The message is now carried by the $alpha_s$ subunit which interacts with a membrane-bound enzyme called adenylate cyclase and 'switches' it on. This enzyme then catalyses the synthesis of the secondary messenger—cyclic AMP—which moves into the cell's cytoplasm and proceeds to activate enzymes called protein kinases.

The role of the kinases is to phosphorylate and thus activate enzymes with functions specific to the particular cell or organ in question. For example, a protein kinase would activate lipase enzymes in fat cells, whereas in muscle and liver cells enzymes involved in glycogenolysis and glycogen synthesis are regulated through an even

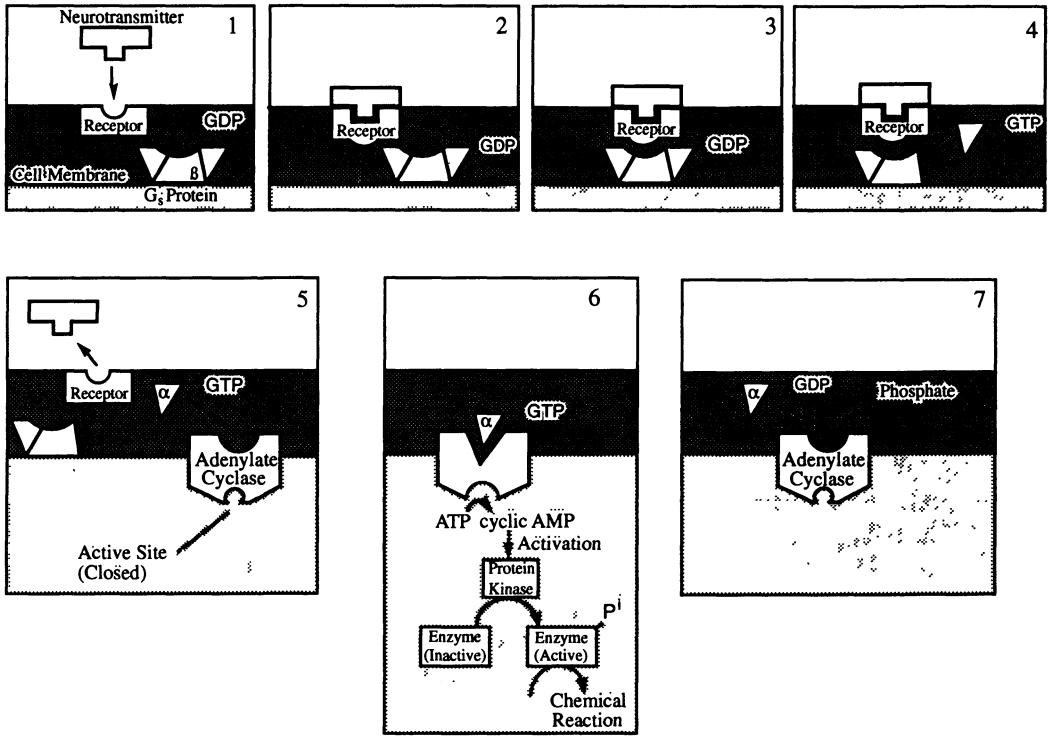


Fig. A3.1 Processes between the combination of receptor and ligand to activation of a target enzyme.

longer sequence of enzymes. How does phosphorylation activate enzymes? Phosphorylation is known to occur on the phenolic group of a tyrosine residue. If this phenolic group is involved in hydrogen bonding, then the addition of a bulky phosphate group disrupts this bonding and also introduces a couple of negatively charged oxygens. These charged groups can now form strong ionic bonds to acidic residues such as aspartate or glutamate and result in the enzyme changing its tertiary structure in order to optimize these new interactions. This change in shape results in the exposure of the active site.

While this is in progress, the G_s protein can be regenerated by the hydrolysis of a phosphate unit from GTP and recombination of the three subunits. The complete process is summarized in Fig. A3.1.

There are several points worth noting about this complicated looking procedure: First of all, the generation of a secondary messenger explains how the original message delivered to the outside of the cell surface can be transmitted to enzymes within the cell and which have no association with the cell membrane.

Secondly, the process involves the switching on of at least three different enzymes —the enzyme synthesizing the secondary messenger, the phosphorylating enzyme (protein kinase A), and the final target enzyme. At each of these three stages, the action of one activating molecule will result in the production of a much larger

number of products. Therefore, the effect of one neurotransmitter interacting with one receptor molecule will result in a final reaction several factors larger. For example, each molecule of epinephrine is thought to generate 100 molecules of cyclic AMP.

Thirdly, there is an advantage in having the receptor, the G_s protein, and adenylate cyclase as separate entities, although this might not be obvious at first sight. Since adenylate cyclase is not intimately associated with a single receptor, the enzyme can be switched on by a variety of different activated receptors and not necessarily of the same type. Therefore, a variety of different receptors can combine with and dissociate the G_s protein since it is the fragmented G_s protein which activates adenylate cyclase.

There is also a G_i protein which is activated by a different set of receptors and has the opposite effect from the G_s protein in that it inhibits adenylate cyclase. As a result, the secondary messenger process is under the dual control of 'brake and accelerator' and this explains the process by which two different neurotransmitters can have opposing effects at a target cell. A neurotransmitter which stimulates the production of a secondary messenger will form a receptor/ligand complex which activates the G_s protein, whereas a neurotransmitter which acts as an inhibitor will fit a different receptor which activates the G_i protein.

It is believed that tolerance to drugs such as morphine (i.e. the necessity to take higher doses to achieve the same result) may be due to some adaptation whereby the receptor/ligand complex is less efficient in activating the G_i protein. As a result, larger levels of drug are necessary.

It has also been proposed that dependence might result from the action of a drug acting through the G_i protein to inhibit the synthesis of cyclic AMP. If this action was prolonged, the system might compensate by increasing the levels of acetylcholine messenger reaching the target cell. This in turn would shift the balance back towards the normal level of cyclic AMP. If the original drug was now removed, there would be an excess of acetylcholine and hence too much cyclic AMP produced, resulting in the observed withdrawal symptoms.

Cyclic AMP is not the only secondary messenger existing in cells. To date, three other secondary messengers have been identified. Cyclic GMP is a secondary messenger synthesized by the enzyme guanylate cyclase by the same mechanism described for cyclic AMP. Two other secondary messengers which have been identified are inositol triphosphate (Fig. A3.2) and diacylglycerol (Fig. A3.3). These are both generated by

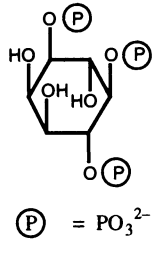


Fig. A3.2 Inositol triphosphate.

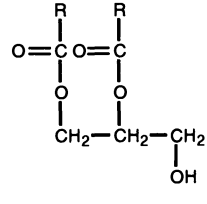


Fig. A3.3 Diacylglycerol.

the process shown in Fig. A3.5. The first part of the process parallels that of cyclic AMP. A neurotransmitter combines with a membrane-bound receptor to form a receptor/ligand complex. This receptor/ligand complex drifts through the cell membrane until it meets a G_s protein. Binding of the receptor/ligand complex with the G_s protein leads to splitting off of the alpha subunit as before. This time however, the alpha subunit goes on to activate an enzyme called phospholipase C. This enzyme catalyses the hydrolysis of phosphatidylinositol diphosphate (PIP_2) (Fig. A3.4) to generate the two secondary messengers inositol triphosphate (IP_3) and diacylglycerol (DG).

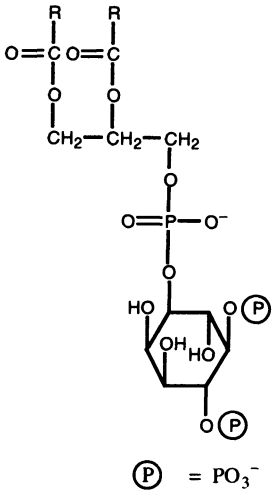


Fig. A3.4 Phosphatidylinositol diphosphate (PIP_2).

The neurotransmitter, receptor, and G_s protein have now completed their part of the process and return to their original states. We now go on to see what the secondary messengers do. Inositol triphosphate is a hydrophilic molecule and moves into the cytoplasm, whereas diacylglycerol is a hydrophobic molecule and remains in the cell membrane (Fig. A3.6). There, it activates a membrane enzyme called protein kinase C (PKC) which catalyses the phosphorylation of enzymes within the cell. Once phosphorylated, these enzymes are activated and catalyse specific reactions within the cell.

Meanwhile, inositol triphosphate is at work within the cell (Fig. A3.7). This messenger works by mobilizing calcium from calcium stores in microsomes or the

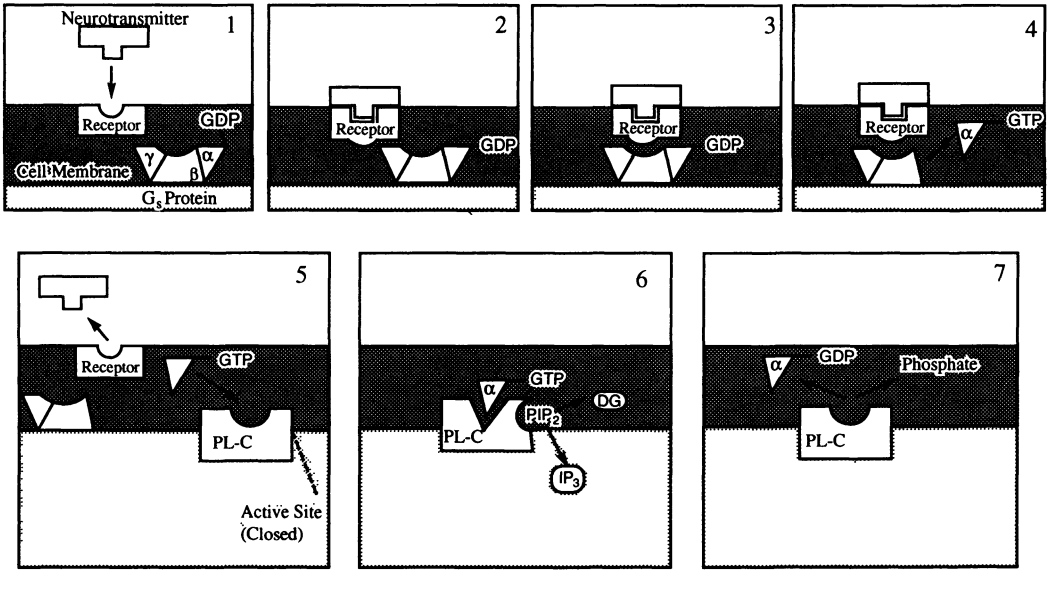


Fig. A3.5 Generation process of inositol triphosphate and diacylglycerol.

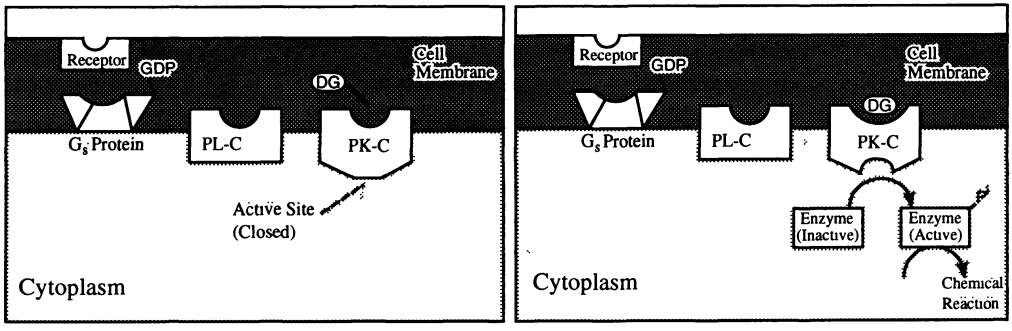


Fig. A3.6 Actions of secondary messengers.

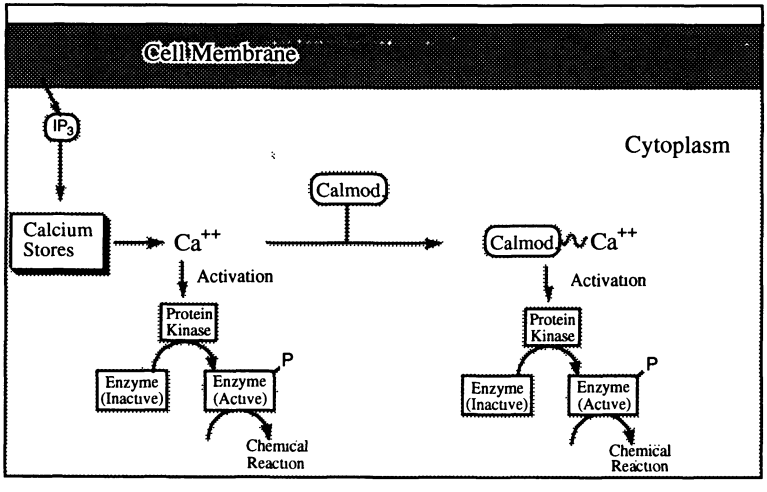


Fig. A3.7 Actions of inositol triphosphate in a cell.

endoplasmic reticulum. Once released, the calcium ions activate calcium-dependent kinases which in turn phosphorylate and activate cell specific enzymes.

The released calcium ions can also bind to a calcium binding protein called calmodulin, which then activates calmodulin-dependent kinases. The overall result is the same with cell specific enzymes being activated.

Once the inositol triphosphate and diacylglycerol have completed their task, they can be linked together again. Oddly enough, they cannot be linked directly and both molecules have to undergo several metabolic steps before resynthesis can occur. It is thought that lithium salts control the symptoms of manic depressive illness by interfering with this involved resynthesis.

Finally, not all chemical messengers bind to their receptors on the outside of the cell, pass on their message and leave. The steroid hormones, for example, do not appear to entrust their message to any of the processes so far mentioned and prefer to deliver their message into the cell in person. The process is believed to be as follows.

The steroid molecule is hydrophobic and is able to diffuse through the cell membrane and finds its receptor waiting for it in the cytoplasm of the cell. Receptor and steroid combine and the complex travels across the nuclear membrane into the nucleus whereupon it binds (still as the receptor/steroid complex) to an acceptor site on the cell's DNA. This binding then switches on transcription and the synthesis of mRNA.

Appendix 4 ▪ Bacteria and bacterial nomenclature

Bacterial nomenclature

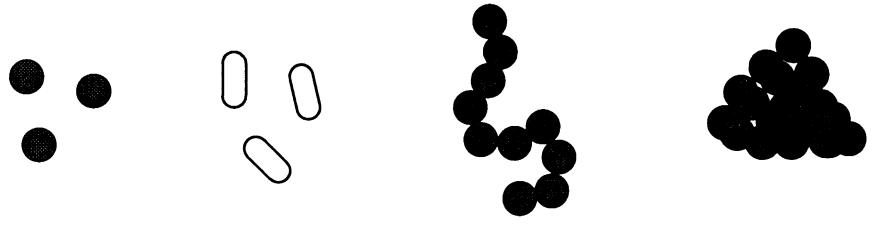


Fig. A4.1 Bacterial nomenclature.

Some clinically important bacteria

Organism	Gram	Infections
Staphylococcus aureus	Positive	Skin and tissue infections, septicaemia, endocarditis, accounts for about 25 per cent of all hospital infections
Streptococcus	Positive	Several types—commonly cause sore throats, upper respiratory tract infections, and pneumonia
Escherichia coli	Negative	Urinary tract and wound infections, common in the gastrointestinal tract and often causes problems after surgery, accounts for about 25 per cent of hospital infections
Proteus species	Negative	Urinary tract infections
Salmonella species	Negative	Food poisoning and typhoid
Shigella species	Negative	Dysentery
Enterobacter species	Negative	Urinary tract and respiratory tract infections, septicaemia
Pseudomonas aeruginosa	Negative	An 'opportunist' pathogen, can cause very severe infections in burn victims and other compromised

		patients, i.e. cancer patients, commonly causes chest infections in patients with cystic fibrosis.
Haemophilus influenzae	Negative	Chest and ear infections, occasionally meningitis in young children
Bacteroides fragilis	Negative	Septicaemia following gastrointestinal surgery

The Gram stain

A staining procedure of great value in the identification of bacteria. The procedure is as follows:

1. Stain the cells purple.
2. Decolourize with organic solvent.
3. Stain the cells red.

This test will discriminate between two types of bacterial cell:

(A) Gram-negative bacteria—these bacteria cells are easily decolourized at stage two and will therefore be coloured red;

(B) Gram-positive bacteria —these resist the decolourization at stage 2 and will therefore remain purple.

The different result in the Gram test observed between these two types of bacteria is due to differences in their cell wall structure. These differences in cell wall structure have important consequences in the sensitivity of the two types of bacteria to certain types of antibacterial agents. It is believed that Gram-negative bacteria have an extra outer layer.

The cells of Gram-positive bacteria have an outer covering or membrane containing teichoic acids, whereas the walls of Gram-negative bacteria are covered with a smooth, soft lipopolysaccharide which also contains phospholipids, lipoproteins, and proteins. This layer acts a barrier and penicillins have to negotiate a limited number of protein channels in order to reach the cell.

Glossary

ADDICTION

Addiction can be defined as a habitual form of behaviour. It need not be harmful. For example, one can be addicted to eating chocolate or watching television without suffering more than a bad case of toothache or a surplus of soap operas.

AGONIST

A drug producing a response at a certain receptor.

ANTAGONIST

A drug which interacts with a defined receptor to block an agonist.

ANTIBACTERIAL AGENT

A synthetic or naturally occurring agent which can kill or inhibit the growth of bacterial cells.

ANTIBIOTIC

An antibacterial agent derived from a natural source (e.g. penicillin from penicillium mould).

BACTERIOSTATIC

Bacteriostatic drugs inhibit the growth and multiplication of bacteria, but do not directly kill them (e.g. sulfonamides, tetracyclines, chloramphenicol).

BACTERICIDAL

Bactericidal drugs irreversibly damage and kill bacteria, usually by attacking the cell wall or plasma membrane (e.g. penicillins, cephalosporins, polymyxins).

BIOISOSTERE

A chemical group which can replace another chemical group in a drug without affecting the biological activity of the drug.

DEPENDENCE

A compulsive urge to take a drug for psychological or physical needs. The psychological need is usually why the drug was taken in the first place (to change one's mood) but physical needs are often associated with this. This shows up when the drug is no longer taken leading to psychological withdrawal symptoms (feeling miserable) and physical withdrawal symptoms (headaches, shivering, etc.) Dependence need not be a serious matter if it is mild and the drug is non-toxic (e.g.

dependence on coffee). However, it is a serious matter if the drug is toxic and/or shows tolerance. Examples: opiates, alcohol, barbiturates, diazepams.

ED50

The ED50 is the mean effective dose of a drug necessary to produce a therapeutic effect in 50 per cent of the test sample.

ISOSTERE

A chemical group which can be considered to be equivalent in size and behaviour to another chemical group; for example, replacing a methylene group with an ether bridge (CH_2 for O).

LD50

The LD50 is the mean lethal dose of a drug required to kill 50 per cent of the test sample.

PARTIAL AGONIST

A drug which acts like an antagonist by blocking an agonist, but retains some agonist activity of itself.

RECEPTOR

A protein in the cell membrane of a nerve or target organ with which a transmitter substance or drug can interact to produce a biological response. Example: cholinergic receptors at nerve synapses.

THERAPEUTIC INDEX (OR RATIO)

The therapeutic index is the ratio of a drug's undesirable effects with respect to its desirable effects and is therefore a measure of how safe that drug is. Usually this involves comparing the dose levels leading to a toxic effect with respect to the dose levels leading to a therapeutic effect. The larger the therapeutic index, the safer the drug.

To be more precise, the therapeutic index compares the drug dose levels which lead to toxic effects in 50 per cent of cases studied, with respect to the dose levels leading to maximum therapeutic effects in 50 per cent of cases studied. This is a more reliable method of measuring the index since it eliminates any peculiar individual results.

TOLERANCE

Repeat doses of a drug may result in smaller biological results. The drug may block or antagonize its own action and larger doses are needed for the same pharmacological effect.

Alternatively, the body may 'learn' how to metabolize the drug more efficiently. Again larger doses are needed for the same pharmacological effect, increasing the chances of toxic side-effects.

Examples: morphine, hexamethonium.

Further reading

Albert, A. (1987. *Xenobiosis*. Chapman and Hall.

Bowman, W. C. and Rand, M. J. (1980). *Textbook of pharmacology*. Blackwell Scientific.

Mann, J. (1992). *Murder, magic, and medicine*. Oxford University Press.

Nogrady, T. (1988). *Medicinal chemistry: a biochemical approach*. Oxford University Press, New York.

Roberts, S. M. and Price, B. J. (ed.) (1985). *Medicinal chemistry—the role of organic chemistry in drug research*. Academic Press.

Sammes, P. G. (ed.) (1990). *Comprehensive medicinal chemistry*. Pergamon Press.

Silverman, R. (1992). *The organic chemistry of drug design and drug action*. Academic Press.

Smith, C. M. and Reynard, A. M. (1992). *Textbook of pharmacology*. W. B. Saunders.

Sneader, W. (1985). *Drug discovery: the evolution of modern medicine*. Wiley.

Stenlake, J. B. (1979). *Foundations of molecular pharmacology*. Volumes 1 and 3. Athlone Press.

Suckling, K. E. and Suckling, C. J. (1980). *Biological chemistry*. Cambridge University Press.

Wolff, M. E. *Burger's medicinal chemistry and drug discovery*. Volumes 1–3. Wiley. (In press.)

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